American Journal of Food and Nutrition, 2020, Vol. 8, No. 4, 101-112
Available online at http://pubs.sciepub.com/ajfn/8/4/1
Published by Science and Education Publishing
DOI:10.12691/ajfn-8-4-1

Hepatoprotective Effects of Mulberries and Cape gooseberry on Thioacetamide Induced Liver Injury in Rats

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Received October 14, 2020; Revised November 15, 2020; Accepted November 24, 2020

Abstract The objective of this study was to evaluate the hepatoprotective effects of black mulberry, white mulberry and cape gooseberry on the thioacetamide (TAA)-induced rats hepatocytes damage in vivo. The data showed that significant differences in the total phenolics and total flavonoids content, between the black mulberry, white mulberry and cape gooseberry. These findings revealed that black mulberry had more total antioxidant activity. The highest levels of glucose, fructose and arabinose were detected in white mulberry. The 54 male *albino* rats used separately into nine groups of 6 rats each group for 4 weeks as follows: Group 1 served as a normal control. Groups 2-9: Rats injected with TAA (100 mg/kg, i.p.) twice a week. Group 2 kept as positive control. Groups 3 and 4 were given 5 and 10 ml/kg b.w rat of black mulberry. Group 5 and 6 were administered 5 and 10 ml/kg b.w rat of white mulberry. Groups 7 and 8 received 5 and 10 ml/kg b.w rat of cape gooseberry. Finally, Group 9 was treated daily with silymarin (100 mg/kg). The effects were compared with a known hepatoprotective agent and silymarin. Alteration in the morphological and the levels of biochemical markers of hepatic damage were studied in the groups. TAA has elevated the liver function, kidney function, lipid profile and MDA levels and reduced the serum levels of albumin, HDL cholesterol, and CAT. Treatments with black mulberry, white mulberry and cape gooseberry juices brought back the altered levels of biochemical markers to the near normal levels at doses independently. Histological examination of the liver tissues confirmed the hepatoprotective effect of fruits juices. These results were documented by the amelioration signs in rat's hepatic architecture. Conclusion: our study demonstrated the ameliorative effects of black mulberry, white mulberry and cape gooseberry juices against TAA induced hepatotoxicity in rats.

Keywords: *black mulberry, white mulberry, cape gooseberry, antioxidants, liver fibrous*

Cite This Article: Hanaa S. M. Abd El-Rahman, Nasra A. Abd-Elhak, and Nahed L. Zaki, "Hepatoprotective Effects of Mulberries and Cape gooseberry on Thioacetamide Induced Liver Injury in Rats." *American Journal of Food and Nutrition*, vol. 8, no. 4 (2020): 101-112. doi: 10.12691/ajfn-8-4-1.

1. Introduction

Liver fibrosis represents a significant global health-care problem and is that the outcome of the many chronic liver diseases, cirrhosis, hepatitis, and toxin accumulation [1]. Moreover, liver fibrosis is conjunction with accumulation of extracellular matrix (ECM) proteins, which may be a characteristic of most kinds of chronic liver diseases. Accumulation of ECM proteins distorts the hepatic architecture by pointing a fibrous scar, and thus the following development of nodules of regenerating hepatocytes defines cirrhosis as provoking hepatic failure or hepatocarcinoma [2]. Liver fibrosis is assumed to be a process of hepatic parenchyma and collagen collapse, and it's going to happen as results of some etiological factors like alcohol consumption, obesity, metabolic disorders, cholestasis, steatosis, viral infection, and toxin aggregation [3]. Thus, thioacetamide (TAA) is taken into account a hepatotoxin causing centrilobular necrosis [4], also induce apoptosis and periportal inflammatory cell infiltration in rat liver [5]. Thioacetamide (TAA) causes an altitude of oxidative stress, enhancing free radical– mediated damage to proteins, lipids and DNA [6].

Over the years, medicinal plants are found useful within the treatment and management of different health problems. About 80% of the world population relies on the utilization of traditional medicine [7]. Plants are exemplary source of medicines and variety of other drugs is derived directly or indirectly.

Mulberry is that the most medicinally important plant which belongs to genera *Morus*. This plant is distributed in India, China, Japan, Arabia and South Europe. It helps in treatment of the many serious diseases like *Diabetes mellitus*, arthrosclerosis, hyperlipidemia, hypertension. There are some dozen of species found in genus *Morus*. Mulberry may be grown both in tropics and in the temperate regions as reported by Aruna *et al*., [8]. The species *Morus* may be a rich source of phenolic

compounds, including flavonoids and anthocyanins. Mulberry contain a great biological, pharmacological and structural interest because of their antioxidant properties [9,10]. Traditionally, the black and white mulberries are used for the prevention of liver and kidney diseases, joint damage, and anti-aging, because of their antioxidant properties [11]. Many populations use medicinal plants as a therapeutic treatment, because of their lower cost. Among the plant species used for medicinal purposes are those of the genus *Morus*. The foremost known species are *Morus alba, rubra, and nigra*, as reported by Elisana *et al*., [12]. Black and white mulberry plants contain glycopeptides and hydrophobic flavonoids (flavones and flavonone) which play main role in hypoglycemic action. Furthermore, mulberry contains many active compounds polyphenols, carotenoids and vitamin A, C and E [13,14]. Mulberry is demonstrated in protecting liver, improving eyesight, facilitating discharge of urine, lowering of blood pressure, anti-diabetic and controlling weight in human's also animal models. It is the necessity of the hours to explore its medicinal value by Indians Aruna *et al*., [8].

Cape gooseberry (*Physalis peruviana L.*), known in shop locally as harankash (in Egypt) and known in English speaking countries as cape- gooseberry or golden berry, has many medicinal and edible uses as fruit [15]. Also, cape gooseberry contains phenolic compounds, tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides, and emblicol [16]. Cape gooseberry extracts have also been reported to possess hypolipidemic [17], anti-obesity [18], anti-diabetic [19], anti-cancer [20], hepatoprotective, and anti-inflammatory [21] activities. A pilot clinical study showed lowering in the total cholesterol and LDL cholesterol levels, enhancement of beneficial HDL cholesterol levels. The current study was designed to evaluate the hepatoprotective effect of black mulberry, white black mulberry, and cape gooseberry juices on thioacetamide induced hepatotoxicity in rats.

2. Materials and Methods

2.1. Chemicals and Reagents

In this experiment the subsequent was used: thioacetamide (TAA) and Silymarin were purchased from Sigma-Aldrich, USA. Folin-Ciocalteu reagent, gallic acid, anhydrous sodium carbonate, aluminum nitrate, and potassium acetate were purchased from Sigma-Aldrich, Germany. Methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Merck, Germany. Flavonol and phenolic acid standards of HPLC-grade purity and purchased from Sigma-Aldrich (St Louis, MO, USA). All biochemical assay kits were purchased from Randox Laboratories Ltd, Diamond Road Crumlin, Co, Antrim, UK, BT294QY.

2.2. Plant Material

The following non-cultivated and traditionally collected autumn fruits of three species were studied: black mulberry, white mulberry and cape gooseberry. These fruits were bought from the local market at Giza, Egypt.

2.3. Preparation of Juice

Briefly, preparation of juice from fruits (black mulberry, white mulberry and cape gooseberry) were blended for 3 min with a blender (Moulinex, France) as individually. 10 g for white mulberry was given 5 ml juice. On the other hand, 5 ml juiced from 7.65 g for black mulberry. 5 ml cape gooseberry juice was made up of 6.65 g. The juices were taken to the laboratory for analytical assays. Additionally, the juices obtained were given orally to different groups of rats at a dose of 5 and 10 ml/kg body weight rats for black mulberry, white mulberry and cape gooseberry.

2.4. Quantitative Determination of Phytochemicals in Juices

2.4.1. Total Phenolic Content

Total phenolic content (TPC) was determined colorimetrically by the Foline-Ciocalteau method according to Cosmulescu and Trandafir [22]. Absorbance was detected with a spectrophotometer at 765 nm. Results were compared to gallic acid standard and expressed as mg gallic acid equivalents/100 ml juice.

2.4.2. Total Flavonoids Content

Total flavonoids were determined using the aluminium nitrate colorimetric method described by Cosmulescu *et al*., [23]. Absorbance was measured at 510 nm against a blank and results were compared to a catechin standard and expressed as mg of catechin equivalents/100 ml of juice.

2.4.3. Analysis of DPPH Radical Scavenging Activity

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out estimated by Thaipong *et al*., [24]. The antioxidant activity was decided by means of a calibration curve prepared with ascorbic acid, and expressed as mg of ascorbic acid equivalent/ml of sample.

2.4.4. HPLC Analysis of Phenolic and Flavonoid Component

Phenolic and flavonoids concentrations of black mulberry, white mulberry and cape gooseberry were determined by HPLC like the method described Goupy *et al*., [25] and Mattila *et al*., [26], respectively. As follows: 1g of sample was mixed with methanol and centrifuged at 10000 rpm for 10min ((HERMLE Z206A, Germany) and therefore the supernatant was filtered through a 0.2 µm Millipore after that, it injection into HPLC, using equipped with a variable wave length detector (Agilant, Germany) 1100. Also the HPLC was equipped with auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C18 reverse phase (BDS 5 µm, Labio, Czech Republic) packed stainless-steel column (4×250 mm, i.d.), malti wavelength detector set at 330 nm and 280 nm for detection of flavonoids and phenolic compounds, degasser, column used for fractionation Zorbax OD.4.6x250nm and also the flow rate of mobile phase during run was 1 ml/min. The

column temperature was maintained at 35°C. HPLC method started with linear gradient at a flow rate of 1.0 ml / min with mobile phase of water / acetic acid (98: 2 v/v, solvent A) and methanol / acetonitril (50: 50, v/v, solvent B), starting with 5 % B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, 100% at 55 min. The initial condition was re-established by 5 min wash in both solvents.

2.4.5. Determination of Total, Reducing and Non Reducing Sugars

Total, reducing, and non-reducing sugars of black mulberry, white mulberry and cape gooseberry were determined according to A.O.A.C [27].

2.4.6. HPLC Analysis of Sugars Fractionation

Sugars were determined according to the modified method described by Melgarejo *et al*. [28] and Zielinski *et al*., [29]. Briefly, 10 g the fruits was homogenized and centrifuged at 12 000 rpm for two min at 4°C, then the supernatant was filtered and transferred into a vial and used for analysis. Analysis of reducing sugars was performed by the chromatographic system coupled to the index detector refraction (HPLC-RI) was equipped with a quaternary pump (Waters 2695 Alliance, Milford MA, USA), degasser, auto injector, and Waters RI 2414 index refraction detector (Milford MA, USA), and also the chromatographic data were acquired using the Empower 2 software. The samples obtained as described above were analyzed using an ion exchange column under isocratic condition with type 1 water (Milli-Q Integral, Millipore, Sao Paulo SP, Brazil). The injection volume was 10 µL and thus the rate of flow was 0.5 mL min–1. The column temperature was maintained at 80°C and therefore the detector at 50°C. Sample detection was performed by comparing retention time standards.

2.5. Animals

Fifty-four adult male healthy male *albino* rats weighing 200±20 g were utilized in our study. Rats were kept under normal health laboratory conditions and ate basal diet for one week. Water and basal diet were provided *ad libitum* for 30 days, according to Reeves *el al*., [30]. All the experimental procedures were carried out in accordance to the rules of Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. The rats were weighed and recorded weekly throughout the experiment.

2.6. Preparation of Thioacetamide

To induce hepatic fibrosis, TAA (100 mg/kg body weight) was prepared freshly by dissolving in 0.9% w/v saline. Then, it has been administered intraperitoneally (i.p) to the rats twice a week for 4weeks. TAA was chosen in line with a previous study, with some minor modifications [31].

2.7. Preparation of Silymarin

Silymarin as reference drug (100 mg/kg body weight) was dissolved in sterile distilled water for oral administration to rats [32].

2.8. Experimental Design

Following laboratory adaptation, rats were randomly divided into nine groups, each group comprising of six rats. Group 1 (negative control) was injected intraperitoneally (i.p.) with saline (5 ml /kg b.w.) twice a week for 4 weeks. Groups (2-9): Rats injected with TAA (100 mg/kg, i.p.) and therefore the following treatments were given by oral route doses in concomitant with TAA for four weeks. Group 2 kept as positive control. Groups 3and 4 were given 5 and 10ml /kg from black mulberry. Group 5 and 6 were administered 5 and 10 ml/Kg from white mulberry. Groups 7and 8 were received 5 and 10 ml /Kg from cape gooseberry. Finally, Group 9 was treated daily with silymarin (100 mg/kg).

2.9. Serum Biochemical Analysis

At the end of experimental rats were weighed. All rats were fasted for twenty-four hr., and then blood samples were collected from the animal's eye plexus under diethyl ether anesthesia. After that, the animals were sacrificed and livers were rapidly dissected out and fixed in 10% neutral buffered formaldehyde for histopathologic examinations. Serum from blood was separated by centrifugation at 4000 rpm, 10 °C, for 15 min and stored at -18°C until analysis. The liver index was calculated as follows (weight of liver/total body weight x 100). Serum total cholesterol, HDL-cholesterol, and triglyceride were determined by using the methods described by Wastson [33], Wieland and Seidel [34] and Fossati and Prencipe [35], respectively. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by the method of Bergmeyer and Harder [36]. Alkaline phosphatase (ALP) activity was measured at 405 nm by the formation of para-nitrophenol from paranitrophenyl phosphateas a substrate using the method of Varley *et al*., [37]. The activity of catalase (CAT) and lipid peroxidation level (malondialdehyde, MDA) of rat serums were estimated according to Aebi [38], and Ohkawa *et al*., [39]. Total and direct bilirubinwere determined according to Doumas *et al*., [40]. Moreover, the serum level of albumin was determined by Doumas *et al*., [41]. Creatinine and urea were determined by using the methods described by Larsen [42] and Orsonneau *et al*., [43].

2.10. Histopathological Examination

The tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for histological studies. Sections (4 μ thick) were prepared and stained with hematoxylene and eosin [44]. Histopathological examination was done by the histopathological laboratory of Veterinary Medicine Faculty, Cairo University.

2.11. Statistical Analysis

The data obtained was subjected to one way ANOVA and Egypt's multiple comparison tests using SPSS statistical (Version 10). Values were expressed as mean± SE. P value $p< 0.05$ was considered significant [45].

3. Results

The yield fruit extract, antioxidant activity capacity, the total phenolic and total flavonoid content of different fruits are tabulated in Table 1. The results of our research presented in this study showed that the cape gooseberry and black mulberry have the highest yield percentage. While, white mulberry is recorded that the lowest value. All fruits capable of scavenging DPPH radicals exhibited antioxidant activity in the following order: black mulberry >white mulberry > cape gooseberry, as shown in Table 1. The black mulberry and white mulberry showed the highest radical scavenging activity, significantly different than the cape gooseberry fruits. Our data showed that black mulberry had more total antioxidant activity and total flavonoids than cape gooseberry and white mulberry. As results, cape gooseberry contained the highest amounts of total phenolic compounds comparing black mulberry and white mulberry.

In order to quantify and separate phenolic compounds of black mulberry, white mulberry and cape gooseberry (mg/100g) by HPLC analysis was used and the obtained results are summarized in Figure 1. In our study, the content of phenolic compounds were found to be higher in black mulberry and cape gooseberry than white mulberry. Whilst, the major compounds of phenolic were E-vanllic, Cholrogenic, and pyrogallol for black mulberry, cape gooseberry and white mulberry, respectively. On the other hand, E-vanllic was the major component for black mulberry.

The contents of individual flavonoid compounds (mg/100 g) of black mulberry, white mulberry and cape gooseberry are shown in Figure 2. Eleven flavonoid compounds (luteoin, naringin, rutin, hespirdin, rosmarinic, quercetrin, quercetin, hespirtin, kaempferol, apegenin and 7-hydroxy flavone) were identified and quantified. The current study found that differences in terms of flavonoids between the analyzed fruits. Hespirdin was the highest followed by rutin in fruit species black mulberry, white mulberry and cape gooseberry.

The total, reducing and non-reducing sugar contents of the selected fruit samples are given in Table 2. There were statistical differences in terms of the total, reducing and non-reducing sugar contents among fruits in the present study. The white mulberry found to has a higher amount of total and reducing sugars, while the lower values were found for cape gooseberry. Whereas, non-reducing sugar level of in the black mulberry was elevated. As seen from these results, the fewest total, reducing, and non-reducing sugar compounds were contained in the cape gooseberry.

In this study, the values of glucose, fructose, sucrose, arabinose, glucuronic, xylose, galacturonic, manitol, mannose and L-rhaminose were estimated in the fruits of black mulberry, white mulberry and cape gooseberry by HPLC (Figure 3). It is remarkable that, white mulberry (light colored mulberry) have high glucose and fructose content than black mulberry (dark colored). However, cape gooseberry has the lowest levels of these sugars.

Table 1. Yield extract, DPPH free radical scavenging activity, total phenolic and total flavonoids contents of different fruits

Name of fruit	Yield % (based on fresh weight)	DPPH activity (mg AAE*/ml)	Total phenolic content as gallic acid (mg/100 ml juices)	Total flavonoids content as catechin (mg/100 ml juices)
Black mulberry	65	8.48±0.458 [a]	0.71±0.125 [b]	1.06±0.364 [a]
White mulberry	50	7.87±0.125 [a]	0.60±0.564 [c]	0.43±0.125 [c]
Cape gooseberry	75	2.16±0.325 [b]	0.89±0.145 [a]	0.77±0.150 [b]

Results are mean values of three determinations ± standard Error. Values sharing the same letter in a line are not significantly different (P≤0.05). AAE*=ascorbic acid equivalent.

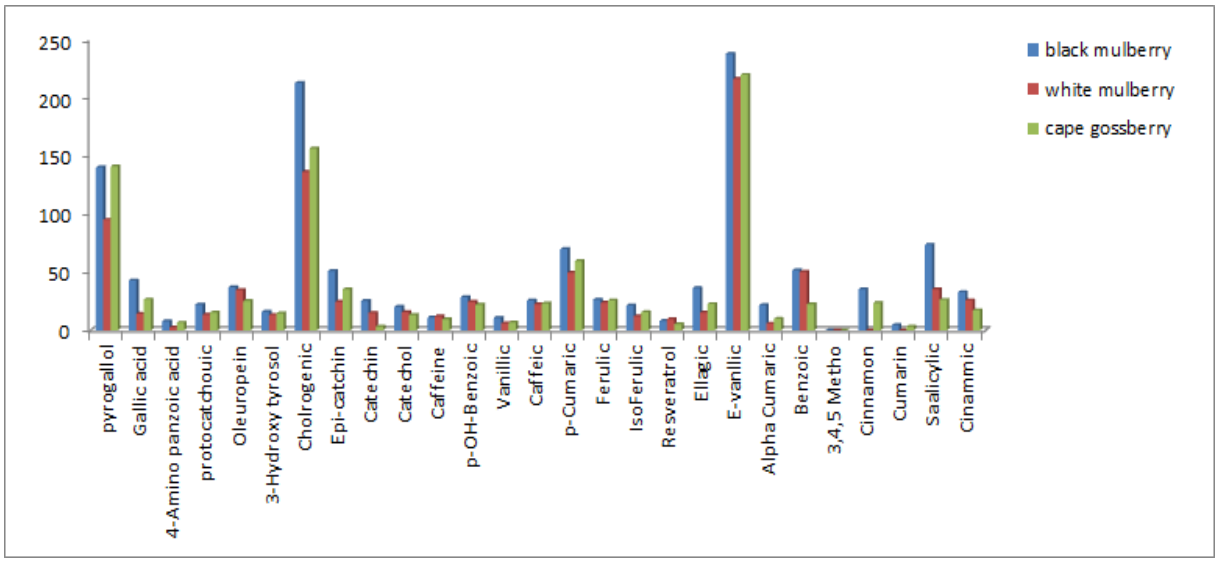

Figure 1. Concentrations of phenolic compounds (mg/100g) of black mulberry, white mulberry, and cape gooseberry by HPLC fractionation

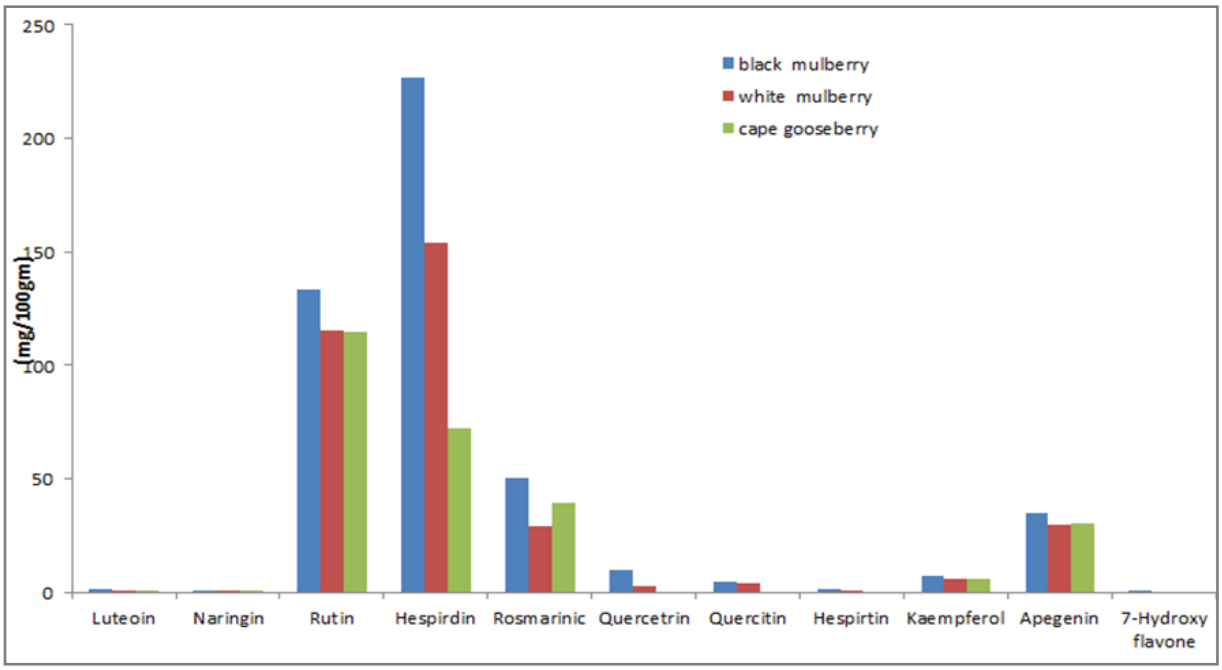

Figure 2. Flavonoid compounds (mg/100g) of black mulberry, white mulberry and cape gooseberry

Table 2. Total, reducing, and non-reducing sugars content of different fruits as g/100gm fresh weight

Content	Reducing sugar	Non reducing sugar	Total sugar
Black mulberry	2.48[b]	3.08[a]	5.56[b]
White mulberry	5.90[a]	1.65[b]	7.55[a]
Cape gooseberry	1.39[c]	0.06[c]	1.45[c]

Results are mean values of three determinations ± standard Error. Values sharing the same letter in a line are not significantly different (P≤0.05).

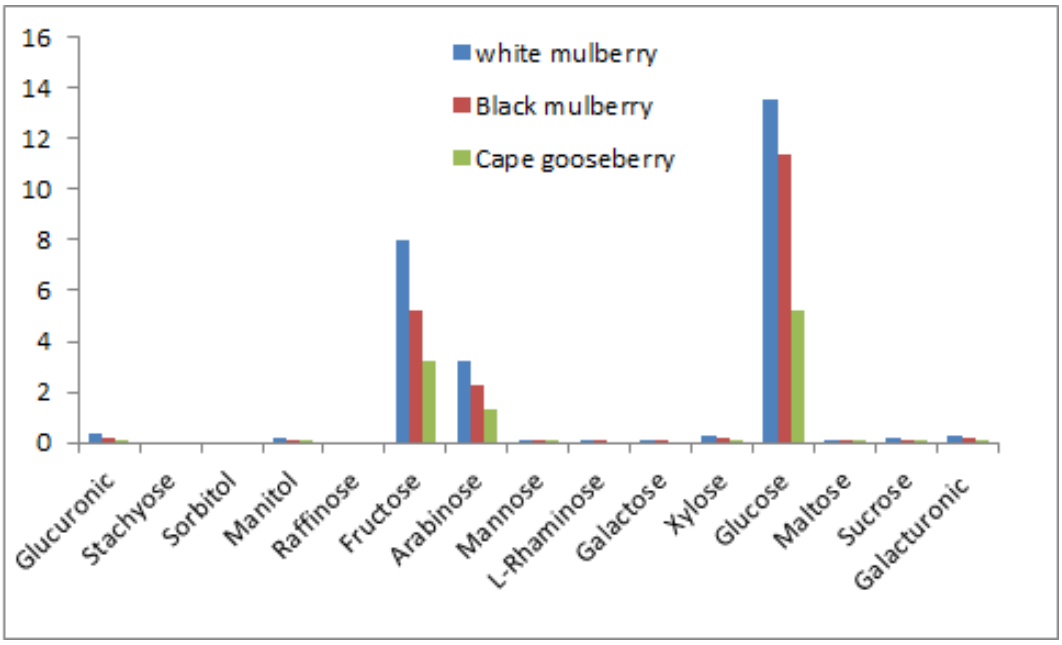

Figure 3. The total sugar compounds of different fruits (%)

3.1. *In vivo* Hepatoprotective Activity

TAA administration rats exhibited severe liver failure. As evidenced by changes in biochemical parameters and morphological architecture. Biochemical parameters such as AST, ALT, ALP and total and direct bilirubin were significantly increased in group 2 which animals treated with thioacetamide when compared to control group (Table 3). However, these increases were markedly alleviated by administration of black mulberry, white mulberry, cape gooseberry, and silymarin in TAA-induced liver fibrosis. The rats which treated with cape gooseberry at dose 10 ml/Kg (G8) was found to be more effective and similar to control group (G1).

Table 3. Effects of black mulberry, white mulberry and cape gooseberry on the serum biochemical parameters in rats

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dl)	
				Total	Direct
G1 control negative	56.8085± 1.83^{a}	27.20±2.361^{a}	177.67±4.333^{a}	1.24±0.196^{a}	0.07±0.006^{a}
G2 control positive	185.496±0.511^{e}	148.41±0.700^{e}	455.67±20.69^{e}	7.15±0.574^{b}	1.60±0.258^{d}
G3 TAA+black mulberry 5 ml/Kg	76.821±0.817^{d}	59.21±0.602^{d}	327.33±12.99^{d}	0.70±0.057^{a}	1.10±0.088^{c}
G4 TAA+black mulberry 10 ml/Kg	66.700±1.44b^{c}	49.51±0.642^{c}	249.33±13.283^{bc}	0.83±0.0152^{a}	0.93±0.124^{c}
G5 TAA+white mulberry 5 ml/Kg	73.756±2.697^{d}	46.16±0.872^{bc}	285.33±19.539^{cd}	0.72±0.035^{a}	1.10±0.205^{c}
G6 TAA+white mulberry 10 ml/Kg	61.806±2.049^{ab}	43.82±0.563^{b}	288.67±13.383^{cd}	0.82±0.014^{a}	0.35±0.121^{ab}
G7 TAA+ cape gooseberry 5 ml/Kg	75.486±2.42^{d}	58.50±0.686^{d}	258.33±23.680^{bc}	0.67±0.003^{a}	0.67±0.179^{bc}
G8 TAA+ cape gooseberry 10 ml/Kg	56.65±1.64^{a}	42.96±0.914^{b}	215.67±13.119^{ab}	0.76±0.003^{a}	0.21±0.066^{a}
G9 TAA+Silymarin 100 mg/kg	68.196±0.763^{c}	45.70±1.767^{b}	295.00±8.660^{cd}	1.20±0.154^{a}	1.02±0.033^{c}

Data presented as mean±SE; the mean values with different superscript alphabets indicate significant differences (P<0.05).

The TAA effect on albumin, kidney function and liver index are shown in Table 4. Remarkably, the control positive was presented the highest levels of creatinine, urea, and liver index, which decreased significantly when black mulberry, white mulberry, cape gooseberry, and silymarin were administered. On the other hand, our results showed that the serum level of albumin was significantly decreased in the group 2 compared with the other groups. According to the obtained results, there were non-significantly differences of albumin level between group 8 which rats received cape gooseberry 10 ml/Kg and group 9 which rats received silymarin 100 mg/kg (standard drug).

The presented data in Table 5 showed that the rats treated with TAA only (G2) had significantly higher levels of serum total cholesterol, LDL-cholesterol and triglyceride than the normal rats (G1). In contrast, the level of HDL-cholesterol was reduced by TAA treatment (G2). The findings show the treatment by black mulberry, white mulberry, cape gooseberry, and silymarin produced a significant reduction in serum total cholesterol, LDL-cholesterol and triglyceride levels and elevated the levels of HDL-cholesterol. Moreover, there were no significant differences in the serum total cholesterol, LDL-cholesterol and triglyceride levels between rats treated with black mulberry (10 ml/Kg,G4) and control group (G1). Our results demonstrated that the capacity of the black mulberry, white mulberry and cape gooseberry juices to control the abnormal lipid profiles hepatic initiated by TAA.

Table 4. Comparison between the control and experimental groups as regards the studied parameters

Group	Albumin (g/dl)	Creatinine (mg/dL)	Urea (mg/dL)	Liver Index (%)
G1 control negative	4.50±.058^{a}	0.56±0.0196^{ab}	46.20±2.055^{a}	1.89±0.013^{a}
G2 control positive	2.297±.144^{e}	0.99±0.0033^{d}	147.00±6.082^{e}	3.03±0.149^{c}
G3 TAA+black mulberry 5 ml/Kg	3.767±.033^{b}	0.78±0.0284^{c}	76.07±2.890^{d}	2.09±0.177^{ab}
G4 TAA+black mulberry 10 ml/Kg	3.267±.067^{d}	0.74±0.0338^{c}	50.60±2.605^{ab}	2.04±0.18^{ab}
G5 TAA+white mulberry 5 ml/Kg	3.767±.033^{b}	0.61±0.005^{b}	80.47±1.705^{d}	2.40±0.14^{b}
G6 TAA+white mulberry 10 ml/Kg	3.867±.033^{b}	0.57±0.005^{ab}	58.40±4.619^{bc}	2.03±0.094^{ab}
G7 TAA+cape gooseberry 5 ml/Kg	3.60±.058^{bc}	0.54±0.0057^{a}	81.03±1.898^{d}	2.11±0.133^{ab}
G8 TAA+cape gooseberry 10 ml/Kg	3.40±.0578^{cd}	0.52±0.008^{a}	53.70±2.119^{ab}	2.01±0.055^{ab}
G9 TAA+Silymarin 100 mg/kg	3.33±0.196^{cd}	0.62±0.0315^{b}	65.97±2.027^{c}	2.04±0.036^{ab}

Data presented as mean±SE; the mean values with different superscript alphabets indicate significant differences (P<0.05).

Table 5. Effects of black mulberry, white mulberry, and cape gooseberry on lipid profiles biomarkers.

Group	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
G1 control negative	96.00±5.131^{ab}	102.67±0.666^{a}	143.00±7.505^{ab}	68.33±2.905^{a}
G2 control positive	172.00±0.577^{d}	214.00±0.577^{d}	364.67±2.728^{f}	21.33±2.027^{g}
G3 TAA+black mulberry 5 ml/Kg	102.33±0.333^{b}	117.00±0.577^{b}	164.00±0.577^{d}	55.33±0.333^{cd}
G4 TAA+black mulberry 10 ml/Kg	97.67±0.333^{ab}	107.00±0.577^{a}	141.67±0.881^{ab}	63.00±0.577^{b}
G5 TAA+white mulberry 5 ml/Kg	94.67±3.844^{a}	135.00±0.577^{c}	182.00±4.509^{e}	47.67±0.333^{f}
G6 TAA+white mulberry 10 ml/Kg	90.67±0.333^{a}	117.00±0.577^{b}	150.33±0.333^{bc}	57.33±0.333^{c}
G7 TAA+ cape gooseberry 5 ml/Kg	93.33±0.333^{a}	113.33±0.666^{b}	156.00±1.154^{cd}	50.67±0.333^{ef}
G8 TAA+ cape gooseberry 10 ml/Kg	91.33±0.333^{a}	115.33±4.807^{b}	136.67±0.666^{a}	57.33±0.333^{c}
G9 TAA+Silymarin 100 mg/kg	111.67±0.881^{c}	119.03±3.064^{b}	178.00±4.0^{e}	52.67±1.452^{dc}

Data presented as mean±SE; the mean values with different superscript alphabets indicate significant differences (P<0.05).

Figure 4 and Figure 5 show the effect of black mulberry, white mulberry, cape gooseberry and silymarin on level of MDA and the activity of antioxidant enzyme CAT in the experimental rats. In group 2 which rats received TAA alone, the antioxidant enzyme CAT was found to be significantly decreased, while serum MDA level significantly increased in comparison with the normal control group.

However, administration of black mulberry, white mulberry, cape gooseberry and silymarin in TAA-induced rats have significantly modified to the above changes by regulating the MDA level which subsequently increased the activity of CAT, and returning to levels near to controls. Among the all doses of juices, 10 ml/Kg from cape gooseberry (G8) showed maximum enhancing effect in the activity of CAT, which was almost comparable to those detected in the control group (G1) and silymarin-treated animals(G9) (Figure 5). Moreover, there were no significant changes in the serum MDA between 10 ml/Kg from black mulberry (G4), 10 ml/Kg from white mulberry (G6), and control group (G1) (Figure 4). These results suggest that treatment with may protect hepatic cells from further damage.

3.2. Histological Analysis of Liver Tissue

Examination was performed on liver obtained 30 days after TAA injection to substantiate the biochemical findings. The histological analysis of liver tissue in the control group (Figure 6a) exhibited normal hepatocytes with well-preserved cytoplasm and nucleus. TAA caused focal necrosis of centrilobular hepatocytes (Figure 6b). Furthermore, the changes were minimal in rats treated with black mulberry, white mulberry, cape gooseberry juices, and silymarin after TAA-induced hepatic injury. The groups G3, G5, G6, G7, and G9 exhibited less slight granularity of the cytoplasm, slight activation of Kupffer cells, and slight fibroplasia in the portal triad. On the other hand, necrosis a more severe form of injury was markedly prevented by black mulberry (G4) and cape gooseberry (G8), respectively (Figure 3).

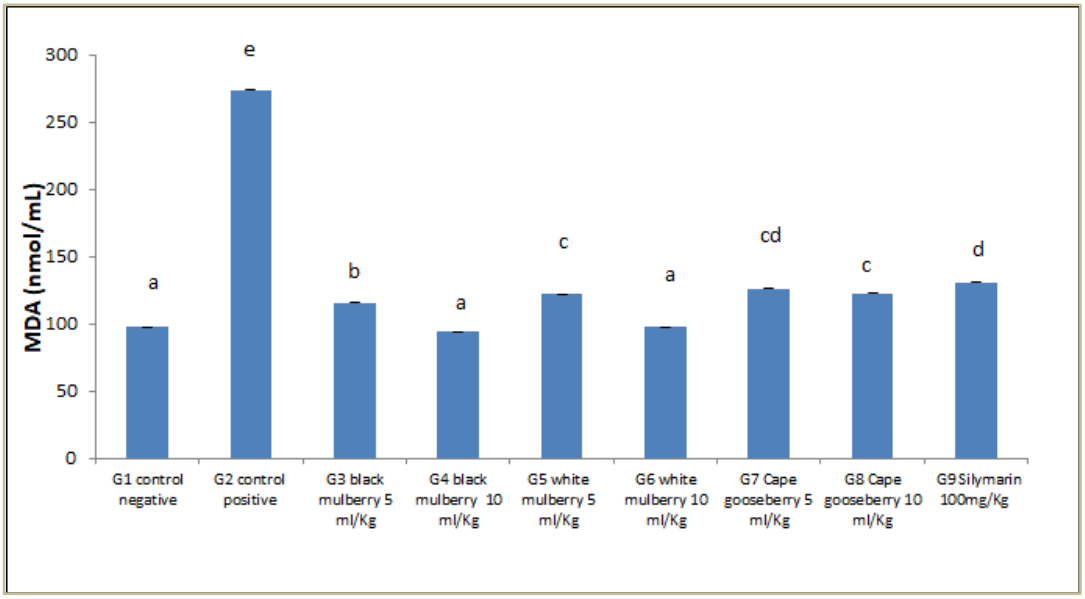

Figure 4. Effect of black mulberry, white mulberry and cape gooseberry on serum MDA in TAA induced liver rats.

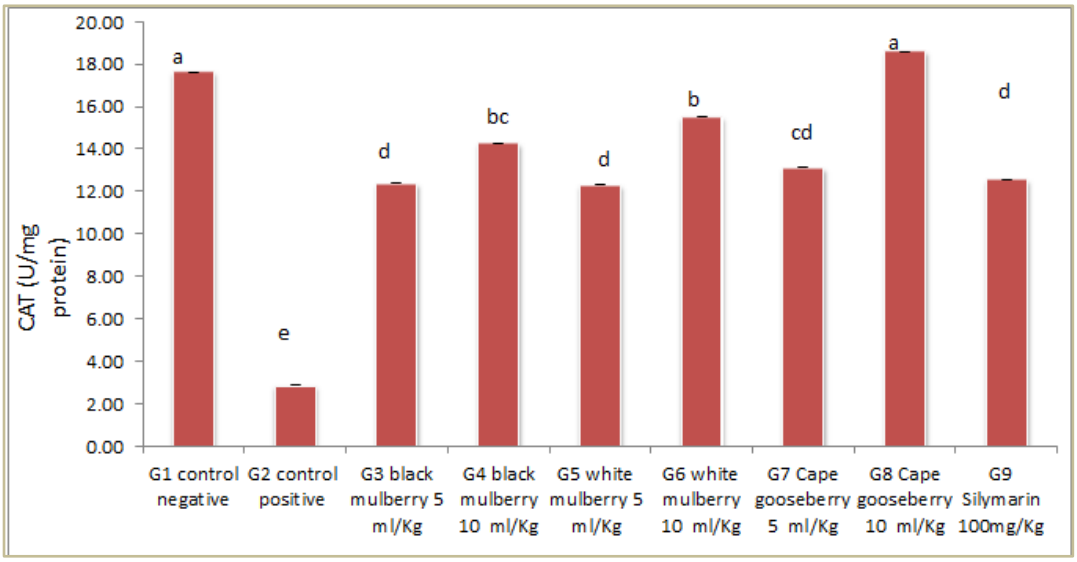

Figure 5. Effect of black mulberry, white mulberry and cape gooseberry on serum CAT activity in TAA induced liver rats

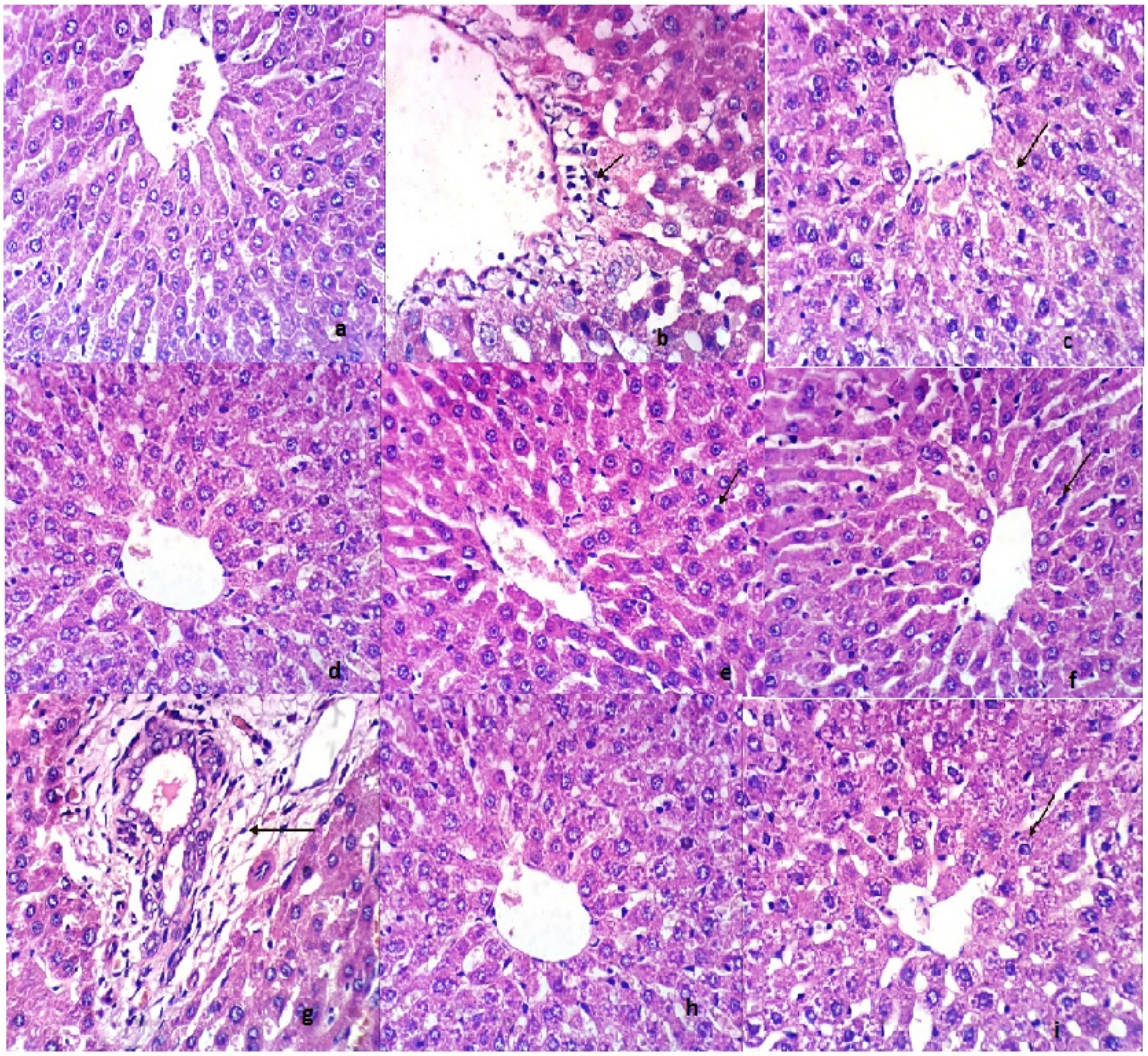

Figure 6. Liver of rat from negative control (G1,a) showing the normal histological structure of hepatic lobule. Liver of rat from positive control (G2,b) showing necrosis of centrilobular hepatocytes. Liver of rat from group (G3,c) showing slight granularity of the cytoplasm of hepatocytes. Liver of rat from (G4,d) showing no histopathological changes. . Liver of rat from (G5, e) showing slight activation of Kupffer cells.Liver of rat from (G6,f) showing slight activation of Kupffer cells.Liver of rat from (G7,g) showing slight fibroplasia in the portal triad. Liver of rat from (G8,h) showing no histopathological changes. Liver of rat from (G9,i) showing slight granularity of the cytoplasm of hepatocytes (H & E X 400)

4. Discussion

Phytochemicals act as important antioxidants, presenting beneficial effects on human health, especially within the prevention of cardiovascular, inflammatory, and cancer diseases. Polyphenols are considered to be amongst the foremost biologically active constituents, contributing greatly to antioxidant activity, total phenolic (TP), total flavonoid (TF) as reported by Polumackanycz *et al*., [46]. The DPPH radical has been widely accustomed to evaluate the radical scavenging ability of varied natural products and has been accepted as a model compound for free of charge radicals [47]. Our data showed that mulberry (white and black) exhibits the stronger antioxidant activity than cape gooseberry. Our results are in agreement with theirs Imran *et al*. [48] and Ramadan *et al*. [49] who reported the high quantity of total phenols was found in whit mulberry 1650 mg/100g, while ,the low content was recorded in black mulberry (880 mg/100g). Chanyotha *et al*., [50] reported that wild Indian cape gooseberry high content of phenolic compounds. Additionally, the wild and kaset Indian cape gooseberries exhibited higher antioxidant activity than ascorbic acid. The results of phenolic compounds identified in several fruit are supported by many authors [51] who they found that the fractionation of phenolic compounds of *Morus nigra* contain phenolic compounds more than *Morus alba*.

Flavonoids, an outsized group of phenolic compounds with activities beneficial to human health, are among several important constituents of edible wild fruits. Within this study showed that flavonoids compounds various a difference between black mulberry, white mulberry and cape gooseberry. A study by Katsube [52] reported that the most flavonol glycoside of white mulberry was rutin. Furthermore, the variation of phenolic compounds within the extract depends on many factors, like the degree of maturity at harvest, genetic differences and environmental conditions during fruit development. A high rutin content and a correlational statistics with rutin results in the conclusion that rutin is especially responsible for DPPH activity. Kobus-Cisowska *et al*., [53] reported that the mulberry fruits may be a valuable source of an entire range of phyto compounds, which final total anti oxidative effect is difficult to predict. Mulberry extract contains large quantities of rutin which is taken into account to be a significant phenolic compound. Indian gooseberry fruits

are utilized in medicinal application [50] as they contain many active ingredients *e.g.* gallic acid, tannins, flavonoids, phenolics and vitamin C. Vitamin C contained in Indian cape gooseberry fruits. In line with our results, the presence of flavonoids (rutin, hispiridin) and phenolic compounds (E-vanllic, Cholrogenic, and pyrogallol) play a crucial role in hepatoprotective.

The current results showed that fructose, glucose and arabinose were the predominant sugars in juices of the different fruits used in this study. These results are in agreement with Sanchez *et al*., [54] who reported that the predominant sugar was fructose (61%) followed by glucose (39%), while sucrose was presented only at trace level in mulberry fruit sampled from spain and they also found big genotypic differences among clones for various sugars, this might flow from to the various environmental and geological conditions.

The liver is that the major metabolic organ and also the biggest glandular a significant within the human body. In the other words, it plays a central role in transforming and clearing the chemicals, but it is susceptible to the toxicity from these agents. Also, liver protects body from the harmful substances [5]. The liver injury occurs due to prolonged exposure with xenobiotic and their metabolites [55]. Exposure to environmental pollutants, chemicals like alcohol, carbon-tetrachloride, thioacetamide (TAA), D-galactosamine, and chronic use of medicine, as an example, paracetamol, rifampicin, isoniazid, etc., can damage the liver cells leading to hepatotoxicity [56]. TAA may be a specific hepatotoxic agent, which induces hepatic damage through generation of reactive oxygen species (ROS), reported by [4]. Within the present study, we planned to research the curative effects of black mulberry, white mulberry, and cape gooseberry juices against TAA-induced hepatoxicity with regard to a typical hepatoprotective silymarin. TAA is an organo sulfur compound, which today is employed as a fungicidal agent and organic solvent within the leather, textile and paper industries [57]. At the same time, it acts as hepatotoxin for the induction of acute hepatic failure. The mechanism by which TAA causes the liver injury involves its biotransformation using the cytochrome P450 enzyme system. These enzymes convert TAA to thioacetamide sulfoxide (TAASO), a reactive intermediate with toxic nature, then to a more reactive thioacetamide-S,S-dioxide ($TAASO_2$), causing severe damage to the liver [58]. Within the current study, administration of TAA at a dose 100 mg/kg twice a week successfully induces hepatoxicity in rats, which confirmed the observation by a previous report [5,57]. According to the biochemical markers and histopathological observations treatment of black mulberry, white mulberry, and cape gooseberry juices demonstrated amelioration of liver injury in TAA-treated rats. Supported our results, there was an interesting alleviate in ALT, AST, ALP, direct and total bilirubin levels of TAA-treated rats with black mulberry, white mulberry, and cape gooseberry juices dose dependently. Furthermore, serum ALT, AST, ALT, and total bilirubin values are serious criteria for the estimation of liver injuries and first markers for a liver function test. Rats induced with TAA experienced hepatic injury manifested by significant changes in serum liver in comparison to other rats. These biomarkers were raised because of systemic damage of the hepatic cells by reactive oxygen species released by TAA. Therefore, the marked release of transaminases and ALT into the circulation indicated severe damage to hepatic tissue membranes during damage liver [59]. Additionally, the above observations explain that these juices have shown clear protection at the cellular level by preventing inflammation in histological studies. Hepatoprotective potential of three fruits we selected in this experimental may be due to presence of flavonoids, phenols and antioxidant activity. They has been documented that flavonoids have very important contribution for hepatoprotective action. Therefore, qualitative investigation of flavonoids was conducted through HPLC. The results of the qualitative investigation revealed the presence of hespirdin, and rutin in black mulberry, white mulberry, and cape gooseberry juices. Due to its edible nature, easy accessibility and economical factor black mulberry, white mulberry, and cape gooseberry can be a good source of active continents having tolerable potential for liver health. This finding is in agreement with the published data by Aftab Ahmad *et al*., [60] and Jantararussamee *et al*., [59].

ALP attributed to the damaged structural integrity of the liver, because of this marker enzyme was cytoplasmic in origin and are released into circulation after cellular damage [61].

In the assessment of liver damage by TAA the determination of kidney function was largely used. In the present study, administration of TAA to rats for 4 weeks has been observed to cause renal toxicity as assessed biochemical parameters these were renal function (urea and creatinine). Rats treated with TAA caused a dramatic elevation in the urea and creatinine. The increasing levels of urea and creatinine show insufficiency of renal function, a similar result has been presented in Table 4. Studies in animals have established that tubular injury plays a central role within the reduction of glomerular filtration rate in acute tubular necrosis. Two major tubular abnormalities could be involved within the decrease in glomerular function in TAA treated rats: obstruction and back leak of glomerular filtrate. The alterations in glomerular function in TAA treated rats may also be secondary to ROS (reactive oxygen species) as described earlier [62] which induce meningeal cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate [63]. On the other hand, the TAA-treated groups showed a significant elevation in liver index due to significantly increased liver weight and decreased body weight compared to control rats, but treatment with black mulberry, white mulberry, and gooseberry juices reversed these defects. Hassan *et al.*, [17] showed that the lowest uric acid, urea and creatinine values recorded for fed rats on 500 mg/kg cape gooseberry.

The decreasing in body weights could be attributed to the toxic effect of TAA throughout the period of the experiment [64]. Furthermore, TAA change fatty acid composition in tissues, thus decreasing fatty acid biosynthesis in the liver. Our results indicated that amelioration in serum lipids in rats treated with black mulberry, white mulberry, and cape gooseberry, they are highly related to the regulation of lipid homeostasis by the phytochemicals in these fruits. Also, our results showed

that rats treated with TAA led to significant reduction in albumin levels. Moreover, the reduced serum albumin level might due to reduction of its synthesis by livers which agreed with Hussein *et al*. [65] and Mousa *et al*., [66]. The decline of total protein level might be attributed to the decreasing effect of TAA on serum albumin level [67]. Deterioration of lipid metabolism is usually found with liver toxicity and liver diseases.Subsequently, lipid profiles (TC, HDL, LDL, and TG) were evaluated the assessment and their correlation with the severity of liver damage. Lipid profiles have long been considered as sensitive indicators of hepatic injury, and the results indicate increased levels of TC, LDL, and TG. The results are in agreement with those obtained by [68] who demonstrated that administration of TAA significantly increases TC, LDL, and TG and decreases serum albumin and HDL. Moreover, our data are in consistence with Gangarapu *et al*., [68] who found that rats treated with TAA show increase in serum total cholesterol, LDL-cholesterol and triglycerides as well as a decrease in serum HDL-cholesterol. Our results are parallel with Palanivel *et al*., [69]; Punitha and Rajasekaran [70] and Gangarapu *et al*., [68]. While, our results were seen other natural compounds such as phenols and flavonoid compounds effectively impacted weight loss and inhibiting lipid accumulation. The results from this study are in line with an animal study conducted by Punitha and Rajasekaran [70] and Gangarapu *et al*., [68].

These alterations in liver function biomarkers might be attributed to TAA-induced liver injuries and oxidative stress in hepatic tissues. Elevated lipid peroxidation biomarker (MDA) and decreased antioxidant enzyme levels (CAT) were observed in the present study after TAA administration. Reduced CAT level may indicate an imbalance in free radical levels and hence increase in cellular damage. There is an imbalance between the quantity of free radicals generated and therefore the antioxidant present within the cell in favor of the oxidation. These findings were harmony in with those of Hajovsky *et al*., [58] and Lu *et al*., [5] who showed that TAA increases MDA while it decreases CAT concentration, and this led to hepatocellular damage and losses of functional integrity of hepatic cellular membrane. Administration of black mulberry, white mulberry, and cape gooseberry juices, and silymarin in the current study significantly improved the CAT activity and reduced MDA, together with the histopathological findings, when compared to the TAA group (G2); these were in preference to the higher dose of black mulberry, white mulberry, cape gooseberry juices. These findings indicate that the beneficial effects of black mulberry, white mulberry, cape gooseberry juices, and silymarin within the healing and regeneration of the hepatocytes, and show its antioxidant potential. The antioxidant activity present in black mulberry, white mulberry, cape gooseberry influence lipid oxidation. Additionally, flavonoids, specifically rutin, and hesperidin were high in juices and that they have positive effects on lipid peroxidation, having the ability to decrease the amount of MDA and to increase the activity of CAT.

The results from the studies indicate that silymarin may be a popular herbal it has a beneficial hepatoprotective and has antioxidant potential to treat liver disorders [71,72]. The TAA administration to rats may causes cellular structure changes, interfere with RNA movement from nuclei to the cytoplasm, and reduce the quantity of viable hepatocytes, also as reduce the oxygen intake rate [6]. Additionally, oxidative stress contributes significantly to the pathogenesis of TAA induced hepatitis and cirrhosis [72]. Within the current study, administration with *Morus nigra, Morus alba* have antioxidant activity that increases cell resistance against lipid peroxidation as a results of radical scavenging. Higher CAT levels compared to SOD means extracts of a traditional Uighur medicine Cichorium glandulosum could promote the antioxidant defence system by increasing CAT activity against H_2O_2 and protecting cells against acute toxic liver damage at 24 h after TAA administration [73]. Our findings also show that *Morus nigra*, *Morus alba*, and cape gooseberry juices, and silymarin are an enormous cause increase in hepatic CAT activity and, thus, diminishes the oxidative injury within the liver because of the radical establishment by the action of TAA. Lipid peroxidation is taken into account one among the important characteristics of oxidative stress [57,72,73,74] reported that TAA is strongly hepatotoxic, which could cause liver lobule necrosis just in case of short-term contact and biliary duct proliferation, hepatic fibrosis and cirrhosis.

Histopathological evaluation of liver sections from TAA treated rats revealed deep centrilobular necrosis while G4 and G8 showed clear signs of the preventive action of rats treated with black mulberry (10 ml/Kg) and cape gooseberry (10 ml/Kg) against TAA-induced damage. Taken together, results from this study provide solid evidence indicating the efficacy of black mulberry and cape gooseberry as a promising anti hepatotoxic agent. These cause black mulberry and cape gooseberry have a high antioxidant activity.

However, the histopathological patterns of the livers of remain groups treated with fruits of black mulberry, white black mulberry, cape gooseberry and silymarin showed slight granularity of the cytoplasm, fibroplasia within the portal triad, and activation of Kupffer cells of liver architecture.

In conclusion, the current study showed that juices of black mulberry, white black mulberry, and cape gooseberry hepatoprotective activity against TAA induced liver injury in rats compared with silymarin Which result from existence of E-vanllic, cholrogenic, and pyrogallol, hispiridin and rutin. Moreover, they manifest enhancement of antioxidant capacities, also decreasing oxidative stress.

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