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Comparative Study of *Hibiscus sabdariffa* L. Extract, Pioglitazone and Simvastatin on NAFLD in Rat Model

Mohamad Mahmoud ElGamal [*1], Ahmed Labib Abdul-Kafy [2], Hussein Mahmoud Elbeltagi [3], Salah El-din S. Semary [4], Alaa M. AboSeif [2]

[1] Department of Pharmacology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt
[2] Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[3] Department of Pharmacology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[4] Department of Pathology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt

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*Corresponding author

Email: drmohamad1984@gmail.com

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ABSTRACT

Background: Liver diseases in Egypt has changed from hepatitis C [after virus c eradication] to nonalcoholic fatty liver disease [NAFLD], which is described as an abundance of hepatic fat buildup [>5%] in hepatocytes determined by histology or imaging.

Aim of the study: To evaluate the potential therapeutic benefits of *Hibiscus sabdariffa* L. extract in rats with non-alcoholic fatty liver disease and compare it with the effect of pioglitazone or simvastatin.

Materials and Methods: Ninety male Sprague-Dawley, weighing between 150 and 200 g [eight weeks old], were purchased from the animal house of the Pharmacology Department at the Mansoura University Faculty of Medicine. Throughout the 12 weeks of the study, rats were kept in ordinary temperature, exposed to the daily light-dark cycles, and had a free access to diet tailored according to their groups. Drugs were given from week nine to week twelve.

Results: Administration of high fructose diet increased hepatic TNF-α, MDA, TG and decreased GSH markers which usually associate fatty liver. These changes were confirmed by histopathological changes which revealed alteration of liver structure, hepatocyte ballooning, inflammation and necrotic changes with significant degrees of macro vesicular and micro vesicular steatosis. *Hibiscus* extract, pioglitazone and simvastatin administration improved hepatic markers and ameliorated the pathological changes by decreasing inflammation, ballooning, with partial improvement of steatosis with higher efficacy belonging to simvastatin followed by pioglitazone then *Hibiscus* extract. The combined drugs showed additive ameliorating effect.

Conclusion: *Hibiscus*, pioglitazone and simvastatin attenuated NAFLD in rat model. *Hibiscus* can be used as supplementary to pioglitazone and simvastatin due its safety and the additive effect of their combination.

Keywords: *Hibiscus extract;* NAFLD; Malondialdehyde; Reduced glutathione; Liver triglycerides.

INTRODUCTION

The most prevalent chronic liver disease globally is non-alcoholic fatty liver disease [NAFLD] [1], which is a group of liver diseases not brought on by alcohol use, including cirrhosis, steatohepatitis and hepatic steatosis. [2].

The calyces of *Hibiscus sabdariffa* L. have been used for many years to treat hypercholesterolemia, hypertension, liver and gastrointestinal diseases in traditional medicine across the world [3]. Research on humans and animals has verified its effects and investigated its potential mechanisms.

Pioglitazone binds to the peroxisome proliferator-activated receptor gamma to work as antidiabetic [4]. Additionally, in certain human studies, pioglitazone was found to ameliorate NAFLD [5].

Statins are antihyperlipidemic drugs that work by competitively inhibiting HMG Co-A reductase, which lowers hepatic cholesterol synthesis [6]. Statins may reduce hepatic inflammation, steatosis, and fibrosis and ameliorate NAFLD/NASH, according to a number of animal studies [7]. Furthermore, several human trials demonstrated the protective effect of statins in NAFLD patients [8].

The objective of the current study was to evaluate the therapeutic efficacy of *Hibiscus sabdariffa* L. extract and compare it with statin and pioglitazone in the treatment of induced non-alcoholic steatohepatitis in rats.

PATIENTS AND METHODS

Drugs effects on tumour necrosis factor-Alpha level: Results presented in Figure [1A] showed significant increase in TNF-α level of HFr [88.07±6] ng/g compared to negative control [45.34±3.7] pg/ml. In addition, the administration of *Hib*. extract, Pio. and Sim. produced significant decrease in TNF-α level [76.99±6.5, 75.94±6.4 and 77.89±5.2] pg/ml, respectively, compared with that of HFr fed group [88.07±6]. Furthermore, the administration of *Hib*. extract + Pio. caused significant decrease the level of TNF-α [62.67±2.2] compared to *Hib*. extract and Pio. groups [76.99±6.5 and 75.94±6.4] pg/ml, respectively. Also, the administration of *Hib*. extract + Sim. showed significant decrease by [63.15±4.4] pg/ml as compared to *Hib*. extract and Sim. groups [76.99±6.5 and 77.89±5.2] pg/ml, respectively. Concerning the combination of *Hib*. extract + Pio. + Sim. didn't significant change in TNF-α level [58.22±2.8] pg/ml as compared to *Hib*. extract + Pio. or *Hib*. extract + Sim. groups [62.67±2.2 and 63.15±4.4] pg/ml, respectively.

Drugs effects on malondialdehyde level: Results presented in Figure [1B] showed significant increase in MDA level of HFr [234.6±10.4] nmol/g compared to that of negative control [118.3±8.5] nmol/g. Likewise, the administration of *Hib*. extract, Pio. and Sim. produced significant decrease in MDA level [175.2 ± 8.2, 189.9 ± 19.8 and 204.2 ± 10.8] nmol/g, respectively, compared with that of HFr fed group [234.6 ± 10.4] nmol/g. Additionally, the administration of *Hib*. extract + Pio. caused significant decrease in the level of MDA [160 ± 10.8] nmol/g as compared to Pio. group [189.9 ± 19.8] nmol/g, without significant change compared to *Hib*. extract group [175.2 ± 8.2] nmol/g. Also, the administration of *Hib*. extract + Sim. showed significant decrease in MDA level [182.3 ± 13.1] nmol/g as compared to Sim. group [204.2 ± 10.8] nmol/g without significant change compared to *Hib*. extract group [175.2 ± 8.2] nmol/g. The combination of *Hib*. extract + Pio. + Sim. significantly decreased MDA level by [129.9 ± 5.2] nmol/g as compared to *Hib*. extract + Pio. and *Hib*. extract + Sim. groups, [160 ± 10.8 and 182.3±13.1] nmol/g, respectively.

RESULTS

Drugs effects on tumour necrosis factor-Alpha level: Results presented in Figure [1A] showed significant increase in TNF-α level of HFr [88.07±6] ng/g compared to negative control [45.34±3.7] pg/ml. In addition, the administration of *Hib*. extract, Pio. and Sim. produced significant decrease in TNF-α level [76.99±6.5, 75.94±6.4 and 77.89±5.2] pg/ml, respectively, compared with that of HFr fed group [88.07±6]. Furthermore, the administration of *Hib*. extract + Pio. caused significant decrease the level of TNF-α [62.67±2.2] compared to *Hib*. extract and Pio. groups [76.99±6.5 and 75.94±6.4] pg/ml, respectively. Also, the administration of *Hib*. extract + Sim. showed significant decrease by [63.15±4.4] pg/ml as compared to *Hib*. extract and Sim. groups [76.99±6.5 and 77.89±5.2] pg/ml, respectively. Concerning the combination of *Hib*. extract + Pio. + Sim. didn't significant change in TNF-α level [58.22±2.8] pg/ml as compared to *Hib*. extract + Pio. or *Hib*. extract + Sim. groups [62.67±2.2 and 63.15±4.4] pg/ml, respectively.

Drugs effects on malondialdehyde level: Results presented in Figure [1B] showed significant increase in MDA level of HFr [234.6±10.4] nmol/g compared to that of negative control [118.3±8.5] nmol/g. Likewise, the administration of *Hib*. extract, Pio. and Sim. produced significant decrease in MDA level [175.2±8.2, 189.9±19.8 and 204.2±10.8] nmol/g, respectively, compared with that of HFr fed group [234.6±10.4] nmol/g. Additionally, the administration of *Hib*. extract + Pio. caused significant decrease in the level of MDA [160±10.8] nmol/g as compared to Pio. group [189.9±19.8] nmol/g, without significant change compared to *Hib*. extract group [175.2 ± 8.2] nmol/g. Also, the administration of *Hib*. extract + Sim. showed significant decrease in MDA level [182.3 ± 13.1] nmol/g as compared to Sim. group [204.2±10.8] nmol/g without significant change compared to *Hib*.

extract group [175.2±8.2] nmol/g. The combination of *Hib*. extract + Pio. + Sim. significantly decreased MDA level by [129.9±5.2] nmol/g as compared to *Hib*. extract + Pio. and *Hib*. extract + Sim. groups, [160±10.8 and 182.3±13.1] nmol/g, respectively.

Drugs effects on reduced glutathione level: Results presented in Figure [1C] showed significant decrease in GSH level of HFr [2.9 ± 0.5] mg/g compared to that of negative control [8.21 ± 1] mg/g. Meanwhile, the administration of *Hib*. extract showed insignificant increase [3.82±.0.1] mg/g in GSH level as compared to HFr [2.9±0.5] mg/g this is unlike GSH level in Pio. and Sim. groups which showed significant increase in GSH level by [5.32±1 and 4.33±1] mg/g respectively compared with that of HFr group. On the other hand, the administration of *Hib*. extract + Pio. caused significant increase in the level of GSH [5.94±1] mg/g as compared to *Hib*. extract group [3.82±0.1] mg/g without significant change compared to Pio. group [5.32±1] uM/g. Whereas, the administration of *Hib*. extract + Sim. showed significant increase in GSH level by [5.54±1] mg/g compared to *Hib*. extract and Sim. groups [3.82±.0.1 and 4.33±1] mg/g, respectively. Additionally, the combination of *Hib*. extract + Pio. + Sim. caused significant increase in GSH level by [7.04±0.3] mg/g as compared to *Hib*. extract + Pio. and *Hib*. extract + Sim. groups [5.94±1 and 5.54±1] mg/g, respectively.

Drugs effects on liver triglycerides level: Results presented in Figure [1D] showed significant increase in TG level of HFr [9.38±1.0] g/100g compared to that of negative control [4.61±0.4] mg/g. While the administration of *Hib*. extract didn't result in significant change in TG level [8.81±0.3] g/100 g unlike TG level in Pio. and Sim. which showed significant decrease in TG level by [7.93±1 and 7.69±1] g/100g, respectively compared with that of HFr fed group [9.38±1] g/100g. On the contrary, the administration of *Hib*. extract + Pio. significantly decreased the level of hepatic TG [7.16±0.3] g/100g as compared to *Hib*. extract and Pio. groups [8.81±0.3 and 7.93±1] g/100 g, respectively. In addition, the administration of *Hib*. extract + Sim showed significant decrease in TG level [6.79±0.5] g/100g as compared to *Hib*. extract and Sim. groups [8.81±0.3 and 7.69±1] g/100 g, respectively. The combination of *Hib*. extract + Pio. + Sim. significantly decreased in hepatic TG level [5.75±0.4] g/100 g as compared to *Hib*. extract + Pio. [7.16±0.3] g/100 g without significant change compared to *Hib*. extract + Sim. [6.79±0.5] group. Results of liver enzymes and lipid profile throughout the study duration and comparison between groups were presented in detail in Figure 2.

Histopathological examination of hepatic tissue specimens: Rats fed standard diet for 12 weeks showed normal liver tissues [Figure 3a]. While those fed HFr diet group, showed moderate lobular inflammation "score 2/3" and marked steatosis "score 3/3" [blue arrow]. Feeding rats with high fructose induced NAFLD [Figure 3c]. On the other hand, treating rats with *Hib*. extract improved inflammation, ballooning, but still present moderate steatosis "score 2/3" [blue arrow] in liver samples [Figure 3d]. In addition, rats treated with Pio. less steatosis than that with *Hib*. extract and mild ballooning degeneration [1/2] [blue arrow]. Central vein congestion was also noted [red arrow] [Figure 3e]. Likewise, rats treated with Sim. [Figure 3-f] showed a mild steatosis "score 1/3", moderate lobular inflammation [score 2], and portal inflammation was also noted [blue arrow]. Meanwhile, combined treatment with both of *Hib*. extract and Sim. or Pio. improved steatosis, inflammatory cell infiltration, ballooning of hepatocytes with the best result seen in the three combined where there is only mild steatosis and inflammation [Figures 3g-i].

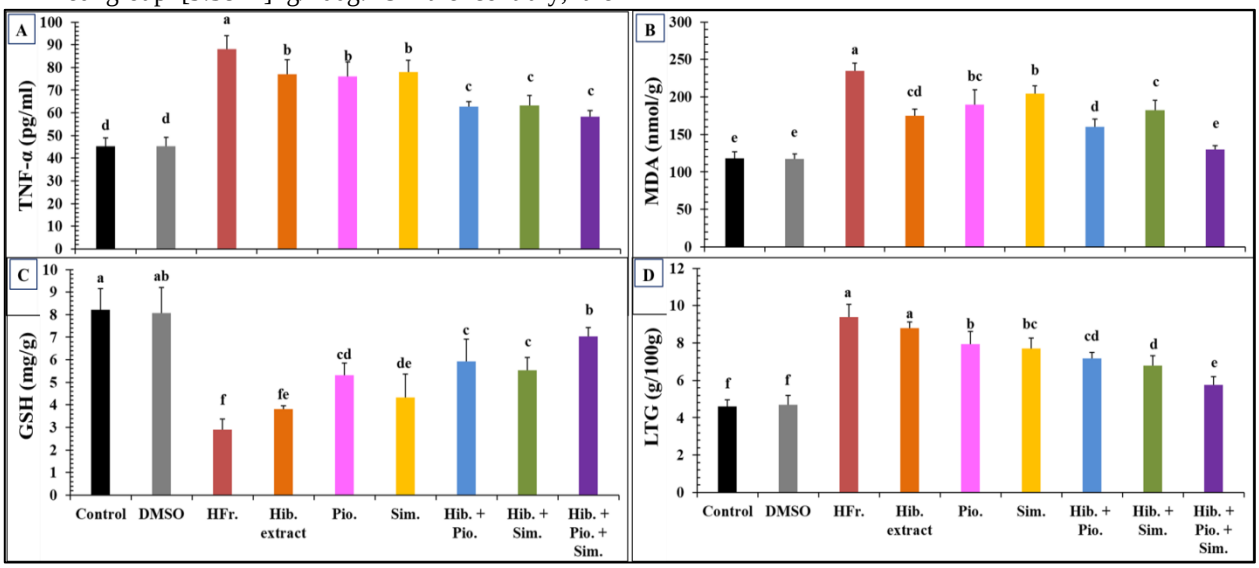

Figure [1]: Effects of *Hibiscus sabdariffa* L. extract [500 mg/kg/day], pioglitazone [5 mg/kg/day] and simvastatin [40 mg/kg/day] alone or in combination for four weeks on [A] tumor necrosis factor-alpha [TNF-α], [B] malondialdhyide [MDA], [C] reduced glutathione [GSH] and [D] liver triglyceride [LTG] levels in liver of fructose treated rats [N: 10/group]. Data expressed as Mean±SD. Abbreviations: HFr.; high fructose, *Hib*.; *Hibiscus sabdariffa* L. extract, Pio.; pioglitazone and Sim. simvastatin

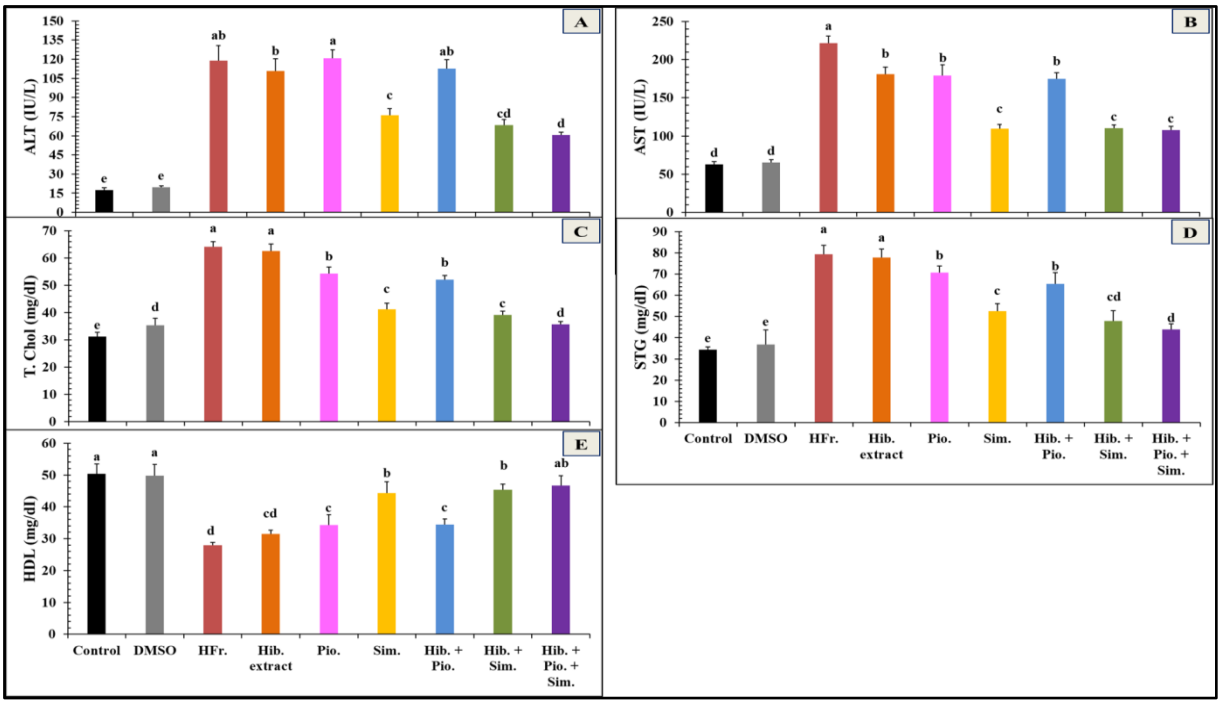

Figure [2]: Effects of *Hibiscus sabdariffa* L. extract [500 mg/kg/day], pioglitazone [5 mg/kg/day] and simvastatin [40 mg/kg/day] alone or in combination for four weeks on [A] serum alanine transaminase [ALT], [B] aspartate transaminase [AST], [C] serum total cholesterol [TC], [D] triglycerides [TG] and [E] high-density lipoproteins [HDL] levels of fructose treated rats [N:10/group]. Data expressed as Mean±SD. Abbreviations: HFr.; high fructose, *Hib.*; *Hibiscus sabdariffa* L. extract, Pio.; pioglitazone and Sim. simvastatin.

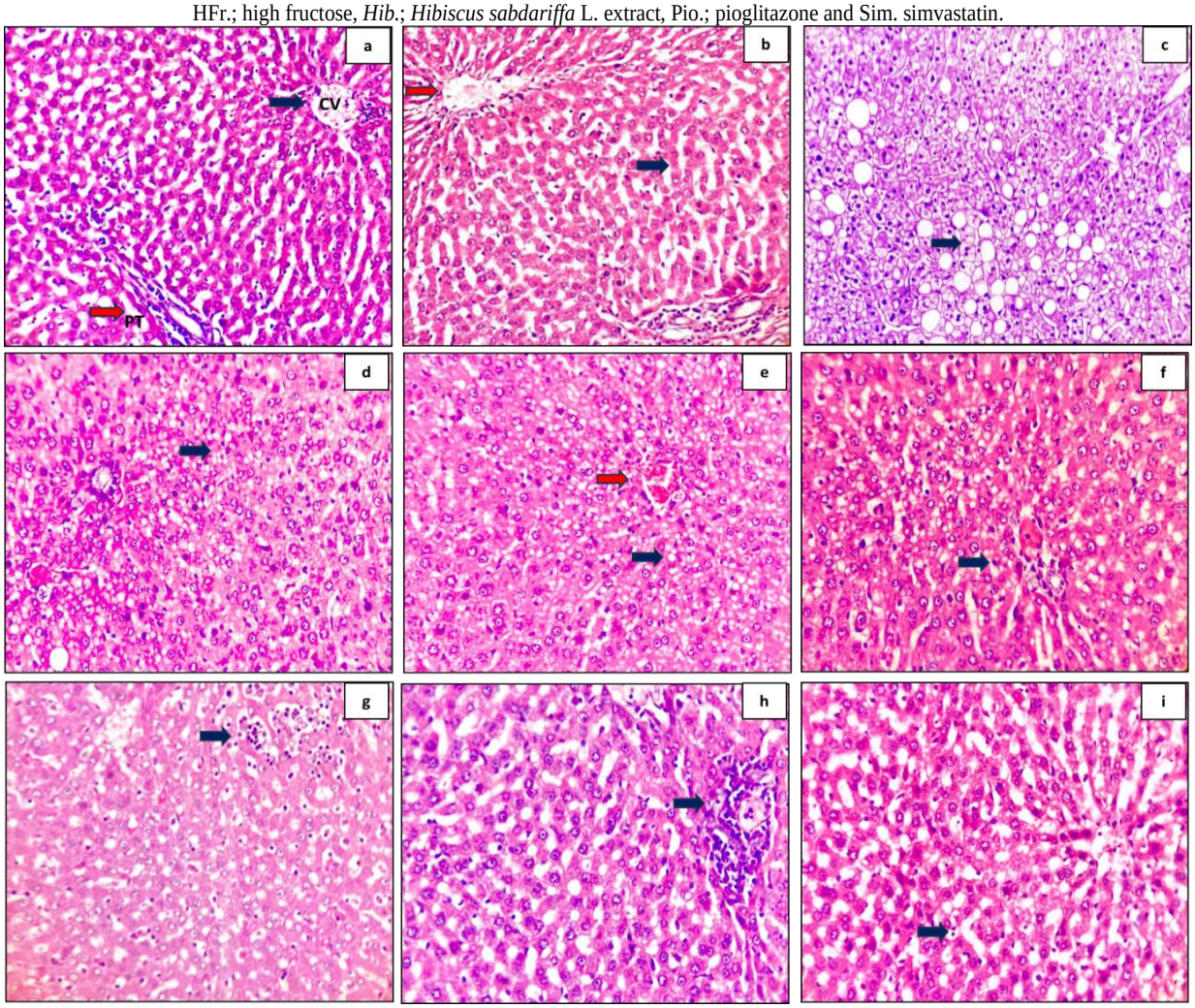

Figure [3]: micrographs of liver sections of rat in different groups: [a] control, [b] DEMSO, [C] HFr, [d] *Hibiscus sabdariffa* extract at 500 mg/kg/day, [e] pioglitazone at 5 mg/kg/day, [f] simvastatin at 40 mg/kg/day, [g] *Hibiscus sabdariffa* extract + pioglitazone, [h] *Hibiscus sabdariffa* extract + simvastatin and [i] *Hibiscus sabdariffa* extract + pioglitazone + simvastatin. [H & E 400x].

DISCUSSION

The frequency of NAFLD has alarmingly increased to 25–40% worldwide. It is currently the primary cause of chronic liver disease globally [19], which puts a tremendous strain on patients and healthcare systems [20]. Only lifestyle intervention is provided as care. The lack of compliance and the fact that there is currently no authorized liver-specific medication therapy with a shown benefit for nonalcoholic fatty liver disease [NAFLD] restrict this strategy. It is critical to create a pharmacological therapy that works [19].

In this study we assessed the effect of *Hib.* extract in treatment of high fructose diet-induced NAFLD in rats and compared this effect with simvastatin and pioglitazone and also combined with these drugs to see the effect of combinations. In addition, in the present study high fructose diet was used to induce NAFLD in male sprague-dawley rats. Feeding rodents a high-fructose diets [> 60%] has been linked to NAFLD development. This is currently one of the most used experimental models [14].

The established NAFLD model was confirmed by measuring the changes in the of biochemical parameters hepatic GSH, MDA and TNF-α as well as serum ALT, AST, total cholesterol, triglycerides and HDL. Also, the changes and comparing them to to the healthy control group. Additionally, in the present study, HFr administration increased serum total cholesterol, triglyceride and decreased HDL level. This in harmony with the results of **Malakul et al.** [21] who demonstrated that giving rats 10.0% fructose in drinking water for 12 weeks elevated levels of serum TC, LDL-C and TG. This is also in parallel with that of **Varghese and Thomas** [22] who found that rats fed on HFr corn syrup had increased total cholesterol and triglycerides and decreased levels of HDL. In addition, in the present study the inflammatory marker TNF-α was also increased in Hfr group this is in agreement with the work conducted by **Abd El-Haleim et al.** [23] who observed an increase in hepatic TNF-α in fructose-induced NASH.

In the current investigation, analysis of the stained liver sections from rats with NAFLD-induced distortions showed necrotic alterations, hepatocyte ballooning inflammation, and liver architecture distortion. Those changes in rats administered a high fructose diet make their NAFLD activity score [NAS] system significantly higher than when rats were fed a normal diet which is in accordance with the report of **Armutcu et al.** [24] who used male Wistar albino rats and gave them 10% fructose dissolved in water for ten days, the rats developed hepatic steatosis, but no inflammation.

In the present study, *Hibiscus* extract decreased AST level and ALT in fructose-induced NAFLD. This is in accordance with the result of **Prasomthong et al.** [16] who observed decrease in AST and ALT levels by *Hibiscus* in liver injury induced by high fat diet. Also, these results are in parallel with that of **Ujianti et al.** [25] who found that *Hibiscus* decreased AST and ALT levels in vit B_{12} deficiency induced steatohepatitis. Morever, these results are in agreement with the report of **Ubani et al.** [26] who reported that extract of *H. sabdariffa* decreased AST and ALT level in phenobarbitone induced rats. Moreover, in the present study, pioglitazone decreased serum AST level in fructose-induced liver injury this is in agreement with the work of **Abdul-Kafy et al.** [17] who noticed decrease in AST level in pioglitazone administration for 7 weeks to NAFLD induced rats. Also, in accordance with the present study **Yaghoubi et al.** [27] found that pioglitazone decreased AST level in randomized clinical trial study that showed the beneficial effects of pioglitazone administration in patients with fatty liver. Furthermore**, Azmi et al.** [28] in parallel to the results of this study found that administration of pioglitazone at a dose of 10 mg/kg BW/day has improved liver functions of male Sprague-Dawley rats with induced fatty liver. Also, in the present study, simvastatin decreased AST and ALT levels in fructose-induced liver injury this is in agreement with the results of **Wang et al.** [29] who showed that simvastatin administration decreased serum AST and ALT levels in rats with non-alcoholic ssteatohepatitis-related liver fibrosis. Moreover, in accordance with the results of the present study **Rodrigues et al.** [30] demonstrated that a dose of 4 mg/kg assisted in the reduction of AST and ALT in NASH-induced mice. In parallel with this study **Pastori et al.** [31] showed in a systematic review and metanalysis that NAFLD patients who administered statins have a significantly reduced in liver enzymes which support using statins safely in NAFLD patients.

In the present study, *Hibiscus* didn't significantly change the elevated level of serum cholesterol, serum triglycerides caused by high fructose administration this in consistent with the work of **Onyenekwe et al.** [32] who didn't find significant change in serum cholesterol after 30,60 day of *Hibiscus* administration. This is in harmony with the work of **Mohagheghi et al.** [33] who's result didn't show hypolipidemic effect of *Hibiscus* in clinical human trial. This in contrast to **Ochani and D'Mello** [34] who found that *H. sabdariffa* extract showed a significant decrease in the serum TC, LDL-C, VLDL-C, TG along with an increase in serum HDL-C levels in cholesterol induced hyperlipidemic rats. Additionally, **Prasomthong et al.** [16] attributed the antihyperlipidemic effect roselle extract to microsomal triglyceride transfer protein [MTP] inhibition and enhancing [LDLR] expression. In this regard, MTP plays a pivotal role in lipoprotein synthesis, while, LDLR take of LDL, VLDL and chylomicrone remnants from bloodstream to regulate lipid levels. While, **Mohagheghi et al.** [33] stated that it is unclear how exactly *Hib*. extract effects on lipid profile, with human and animal studies showing varying results. These discrepancies were

attributed to the length of the studies and the amounts of HS. Likewise, in the present study, pioglitazone improved lipid profile by decreasing total cholesterol, triglyceride and increasing HDL level these results is in harmony with that of **Al-Muzafar et al.** [35] who showed that pioglitazone administration decreased serum TC, TG, and LDL levels, and increased HDL levels in rat model of insulin resistance induced by high fat-carbohydrate diet. This is also in agreement with the report of **Abdul-Kafy et al.** [17] who showed decrease in serum TC, TG in induced non-alcoholic fatty liver in rats by pioglitazone administration. In accordance with the present study **Wang et al.** [36] concluded after a meta-analysis of randomized controlled trials that a reduction of blood lipids in NAFLD patients by pioglitazone administration associated with improvement on liver steatosis. Additionally, simvastatin administration improved serum lipid profile by decreasing total cholesterol, triglyceride and increasing HDL levele this is in accordance with the results of **Da Silva Pereira et al.** [37] who showed that simvastatin administration decreased serum TC, TG, LDL and increased HDL in a mouse NAFLD model induced by a high-fat-high-carbohydrate. Furthermore, these results were in accordance with those of **Hassan et al.** [38] who noticed a decrease in serum TG, TC levels by simvastatin administration to rats with high fat diet induced liver steatosis.

In the present study, *Hibiscus* extract ameliorated oxidative stress this is manifested by decreasing hepatic MDA and increasing GSH. This is in accordance with that of **Adeyemi et al.** [39] who showed that treatment of rats with extracts of *H. sabdariffa* increased hepatic GSH level in STZ-induced liver damage in diabetic rats. In addition, **Al-Groom** [40] results showed that *Hibiscus* decrease MDA and increased GSH level in Cyclophosphamide induced hepatotoxicity in rats. Also, pioglitazone improved oxidant/antioxidant markers by decreasing hepatic MDA and increasing GSH this is in consistent with the work conducted by **Surapaneni and Jainu** [41] who noticed that pioglitazone increased hepatic GSH and decreased hepatic MDA in experimental non-alcoholic steatohepatitis. In agreement with the present study, **Shaaban et al.** [42] showed that three weeks of pioglitazone administration reversed hepatic markers of oxidative stress in NAFLD rats. Moreover, simvastatin decreased oxidative stress by decreasing hepatic MDA and increasing GSH this is in harmony with the results of **Da Silva Pereira et al.** [37] who showed that simvastatin decreased hepatic MDA in mice with non-alcoholic fatty liver. This is also in consistency with the report of Miao **et al.** [43] who noticed decreased hepatic MDA level by simvastatin administration in high-fat diet-induced non-alcoholic fatty liver disease in rats.

In the present study, *Hibiscus* decreased hepatic triglyceride content but not to significant level this is in agreement with the work conducted by **Prasomthong et al.** [16] who noticed significant decrease in hepatic triglyceride content by *Hibiscus* administration in a dose dependent manner in hepatic steatosis of rats fed a high fat diet. This is also in parallel with the of report of **Huang et al.** [44] who showed that administration of *Hibiscus extract* reduced hepatic triglycerides in hamsters fed on high fat diet to induce obesity and liver damage. In addition, pioglitazone decreased hepatic triglyceride which is in consistency with **Collino et al.** [45] who showed that hepatic triglyceride was reduced by pioglitazone administration in overweight rats fed on high cholesterol and fructose diet and attributed this effect to suppressors modulation of cytokine signaling [SOCS-3]. This is in parallel with the work of **Ali et al.** [46] who showed that pioglitazone decreased hepatic triglyceride in rats fed a high-fat diet to induce liver steatosis. In addition, **Della Pepa et al.** [47] showed in randomized clinical trial that one year of low dose pioglitazone administration in type 2 diabetic patients improved indirect indices of NAFLD [Hepatic Steatosis Index, Index of NASH and Liver Fat Equation] in agreement with results of the present study. This is in contrast to the report of **Peng et al.** [48] who found that pioglitazone exacerbates hepatic steatosis in obese diabetic KKAy mice by increasing hepatic TG accumulation by stimulation of PPARγ responsive hepatic genes expression that facilitate the uptake of FFA from the blood into liver which may be due to species differences.

In the present study, simvastatin decreased hepatic triglyceride content this is in parallel with the results of **Da Silva Pereira et al.** [37] who showed that simvastatin administration for seven weeks decreased hepatic triglyceride in mice with non-alcoholic fatty liver. This is also in agreement with those of Prasomthong **et al.** [16] who noticed significant decrease in hepatic triglyceride content by simvastatin administration to rats fed a high fat diet for *hepatic* steatosis induction.

In the present study, *Hibiscus sabdariffa* extract reduced pro-inflammatory cytokines TNF-α level in liver tissue significantly compared to the HFr group this is in accordance with the work of **Prasomthong et al.** [16] who showed that *Hibiscus sabdariffa* extract significantly decreased hepatic TNF-α level in rats fed a high fat diet for hepatic steatosis induction. This is also in agreement with the results of **Ujianti et al.** [25] who showed that rosella extract treatment lowered hepatic TNF-α in rat model with vitamin B^{12} deficiency induced steatohepatitis. In addtion, in the present study, pioglitazone decreased hepatic TNF-α this is in parallel with **Xu et al.** [49] who showed that pioglitazone treatment decreased hepatic TNF-α in rats with NAFLD induced by high fat diet. These results were in parallel with that of **Zhao et al.** [50] who showed that pioglitazone administration to rats fed on high-fat and high-calorie diet for NASH induction significantly decreased hepatic TNF-α level and attributed this effect to hepatic COX-2 expression down-regulation. Also, in the present study, simvastatin decreased hepatic TNF-α in

parallel with **Prasomthong et al.** [16] showed that simvastatin significantly decreased hepatic TNF-α level in rats fed a high fat diet for hepatic steatosis induction. This is also in agreement with the report of **Li et al.** [51] who found that simvastatin administration to mice with high-fat diet-induced non-alcoholic steatohepatitis decreased hepatic TNF-α. Statins exert anti-inflammatory, antifibrotic, and proapoptotic activities and could have a relevant role in NASH beside their cholesterol-lowering effects. Many in vitro and in vivo studies showed that statins affect NASH through influencing pro-inflammatory factors, hepatic cells activation, sinusoidal endothelial cells, crown-like structures [52].

In the present investigation, histopathological study of the liver showed that treating rats fed on high fructose diet with *Hibiscus* improved inflammation, and fibrosis with partial improvement of steatosis this is evident by decreased NAS scoring where *Hibiscus* decreased the amount of macro vesicular steatosis. These findings are in parallel with the results of **Prasomthong et al.** [16] who reported that *Hibiscus* ameliorated hepatic steatosis in rats fed on high fat diet by alleviating lipid accumulation through decreasing triglyceride transfer protein and also by inhibiting the de novo lipogenesis proteins, reducing lipid peroxidation, inflammation. In consistency with the present study, **Ujianti et al.** [25] concluded that *Hibiscus* extract reduced the progression of experimentally induced NAFLD in rats fed on vitamin B_{12} deficient diet by inhibiting lipid synthesis in liver cells and lowering various hepatic pro-inflammatory proteins that lead to suppression of liver inflammation. Furthermore, in the present study, pioglitazone improved histological feature of fructose induced NAFLD by decreasing inflammatory infiltrate and macrovascular steatosis which is evident by decreasing NAS scoring. In parallel with the present study**, Ebihara et al.** [53] observed that TZDs exacerbated fatty liver in mouse models but improved it in rat models similar to those in human patients and attributed this discrepancy to different distribution of PPARγ which is the target of TZDs between rats and mice. In agreement with the present study, **Kabel and Borg** [54] showed that administration of pioglitazone markedly improved the histopathology of liver steatohepatitis by decreasing steatosis and inflammatory infiltration. In contrast to the present study, **Van Der Veen et al.** [55] observed that although pioglitazone attenuated hepatic inflammation and fibrosis and reduced the lipid droplets size, this was insufficient to prevent hepatic steatosis in phosphatidyl-ethanolamine N-methyltransferase-deficient mice. They explained this by noting that lipogenic gene expression didn't change so hepatic lipogenesis and TG formation were not affected by pioglitazone treatment.

In the present study, simvastatin has a better effect in improving liver histopathology of NAFLD than *Hibiscus* or pioglitazone this is presented by a better NAS scoring with noted mild lobular inflammation, Mild steatosis and no evidence of ballooning degeneration. In accordance with this study, **Hassan et al.** [38] showed that simvastatin ameliorated liver Steatosis in rat model of high fat diet and found that simvastatin moderately improved the histological structure of the hepatic tissue, but some central veins still dilated and congested. Some hepatocytes appeared vacuolated and with pyknotic nuclei and attributed this beneficial role of simvastatin is to reducing plasma lipids, decreasing lipid droplets accumulation in hepatocytes also simvastatin protects against damage from oxidative stress resulting from dyslipidemia, inhibits free radicals' synthesis and lessens the lipid peroxidation and oxidative stress induced by HFD, with consequent reduction of inflammation and fibrosis**.**

The ameliorating effect of NAFLD associated features was more significant with simvastatin followed by pioglitazone then hibiscus extract meaning simvastatin is more superior than pioglitazone which is also better than *Hibiscus*. By adding *Hibiscus* to either drugs or the tow drugs combined to it the therapeutic effect of the combination was better than each drug alone there is no antagonism and the better results occurred in the group of *Hibiscus* + pioglitazone + simvastatin.

Conclusion: The present study found that treatment of rats with Hibiscus, pioglitazone, and/or simvastatin [either alone or in combination] attenuated fructose-induced fatty liver as demonstrated by improvements in lipid profile, hepatic oxidative stress markers, hepatic inflammatory mediators that contribute to the pathogenesis of NAFLD this is confirmed by histopathological examination and a decrease in liver triglycerides.

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