# Effect of Yellow-Split Chickpeas on Hepatosteatosis-Induced Serum Oxidative Damage in Rats

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**Abstract:** *Hepatosteatosis represents a severe health hazard to humans due to its prevalence in last time. Therefore, the present study was designed to evaluate the effect of phosphatidylcholine (PC), C. arietinum-enriched PUFA (CAP), and its prepared phytosome form (CAPP) against non-alcoholic fatty liver disease (NAFLD) in rats. Forty-eight rats were randomly divided into eight experimental groups. Control groups including normal, PC, CAP, and CAPP. Normal group received normal diet and distilled water for 45 days. Other control groups received normal diet and administered distilled water for 15 days then administered 500 mg/kg b.wt of PC, CAP, and CAPP, respectively for another 30 days. NAFLD group, rats received a high fat diet (HFD) and injected tamoxafene (TAM) (200 mg/kg body weight) intraperitoneally for 15 consecutive days, then administered distilled water for another 30 days. NAFLD + PC, NAFLD + CAP, and NAFLD + CAPP groups received HFD and injected with tamoxafene for 15 days, then administered PC, CAP, and CAPP, respectively for an additional 30 consecutive days. The results revealed that NAFLD elicited oxidative stress which was evident due to the significant increase of s-LPO, and significant decrease of s-GSH, s-SOD, s-GPx, and s-GRD. However, treatment with PC/ CAP/ CAPP ameliorates the oxidative changes caused by NAFLD. CAPP showed the best regimen which may be due to its phytosome form that allows bioavailability of CAP.*

**Keywords:** Non-alcoholic fatty liver disease, serum oxidative stress, *C. arietinum*-enriched PUFA (CAP), CAP-phytosome (CAPP)

## 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most public chronic liver challenging that increased worldwidely with high prevalence of obesity and type - 2 diabetes (Mitra and Chowdhury, 2020). NAFLD or hepatic steatosis is characterized by accumulation of triglycerides in the hepatocytes without a history of alcohol (Mahmoud et al., 2016). Some drugs potentiate the onset of hepatic steatosis via overproduction of reactive oxygen species (ROS) which create a pro-oxidant state that activate stellate cells to produce hepatic fibrosis (Janevski et al., 2011; Aguirre et al., 2014). In fact,NAFLD frequently overexpressed in obese patients (Divella et al., 2019). Furthermore, increased insulin level due to insulin resistance of obese patients inhibits the synthesis of hepatic apolipoprotein β-100 and increase synthesis of hepatic fatty acids. Consequently, the levels of synthesis and stored triacylglycerols increase in liver (Kawano and Cohen, 2013). Unfortunately, the long chain fatty acids are metabolized by beta-oxidation process resulted in lipid peroxidation and production of ROS such as hydrogen peroxide and hydroxyl radicals in liver (Quijano et al., 2015). The increased hepatic lipid peroxidation induces severe hepatocellular degeneration and necrosis ends by generation of more and more ROS due to mitochondrial lesion (Chen et al., 2020). Further, malondialdehyde and 4-hydroxynonenal, the end products of lipid peroxidation, activate proinflammatory cytokines and stellate cell that ends by severe hepatocyte degeneration, necrosis, fibrosis, inflammatory infiltrates, and steatosis (Sánchez et al., 2020).

Animal models of NAFLD reflect correctly both the histopathology and pathophysiology of human NAFLD (Takahash et al., 2012). Tamoxifen (TAM), adjuvant hormonal therapy that has been widely used for many decades as the "gold standard" treatment for patients with breast cancers (Johnston and Cheung, 2018). However, prolonged TAM treatment associated with various complications, including hypoglycemia, hypertriglyceridemia, changes in plasma cholesterol levels and liver diseases such as NAFLD (Lee et al., 2018). It has been shown that 43% of breast cancer patients treated with TAM develop steatosis within the first two years of treatment (Zhao et al., 2014). TAM caused oxidative liver damage due to overproduction of oxygen radical during its metabolism; as it impair β-oxidation process of fatty acids which leads to overgeneration of reactive oxygen species (ROS) (Cole et al., 2010). Currently, the only effective therapy to treat NAFLD is to decrease body weight in obese individuals, improve insulin sensitivity and decrease oxidative damage through the use of antioxidants (Pydyn et al., 2020).

Recent researches favor to use the medicinal plants instead of using chemical compounds to support liver functions and treat diseases in the liver because they are inexpensive and have few side effects compared to chemical compounds (Helal et al., 2011). Therefore, the discovery of nutrients that ameliorate fatty liver is one of the requirements worldwidely (Yoshitomi et al., 2012). Chickpea (*Cicer arietinum* L.) is one of the most important pulse crop that contain a high amount of polyunsaturated fatty acids (PUFA) (66%) (Jukanti et al., 2012). Specifically, it contains linoleic and oleic acids that are known with their anti-fatty liver effects by inhibition of fat deposition in hepatocytes (Daley et al., 2010), (Jukanti et al., 2012). Additionally, *C. arietinum* is cholesterol free (Jukanti et al., 2012), thereby it is difficult to induce fatty liver. The present work utilizes the yellow split-desi chickpea type known as Chana Dal.

One of the most important limitations of herbal extract is their destruction by the digestive secretion. Therefore,

phytosome technology is a new trend of drug delivery system that enhances the bioavailability of herbal agents by protecting them from the digestive destruction and bacterial gut (Rahman et al., 2020). In phytosomes technology, the herbal extract is bound to phosphatidylcholine producing a lipid compatible molecular complex (Bhattacharya and Ghosh, 2008). As the absorption of active constituent(s) is improved, its small dose can produce desired results. Phytosomes are better able to transition from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell, and thus can be used for systemic targeting (Gandhi et al., 2012).

It is interesting in the present study to use the phytosome as a drug delivery system to study the effect of *C. arietinum* extract against hepatosteatosis. Again, the current work selects the novel drug delivery system (Phytosome) rather than conventional drug delivery system (Liposome), as it is better than the later. Where, in the phytosome it is an integral part of the membrane, being the molecules anchored through chemical bonds to the polar head of the phospholipid. This difference results in phytosomes being much better absorbed (Bhattacharya and Ghosh, 2008). Therefore, the present study investigates the potential ameliorative roles of *Cicer arietinum* enriched polyunsaturated fatty acids (CAP) and *Cicer arietinum* enriched polyunsaturated fatty acids phytosome (CAPP) to suppress the NAFLD induced by high fat diet (HFD) and tamoxifen side effects.

## 2. Materials and Methods

**Identification and classification of *Cicer arietinum* (Chickpea)**
The chickpea (Hummus) is a member of the Fabaceae (Leguminosae) family (table 1). The ongoing study worked on the yellow split-desi chickpea (Fig. 1). Seeds were cleaned and freed from broken seeds and other foreign materials. *Cicer arietinum* enriched polyunsaturated fatty acids (CAP) and its phytosome (CAPP) were prepared according to Salama et al. (2018) with some modification and Amit et al. (2013), respectively (Fig. 2).

**Table 1:** Taxonomy of *Cicer arietinum* L.

| Scientific classification | |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Class | Magnoliopsida |
| Order | Fabales |
| Family | Fabaceae |
| Genus | *Cicer* L. |
| Species | *Cicer arietinum* L. |



**Figure 1:** *Cicer arietinum* L. (Yellow split-desi chickpea, Chana Dal).
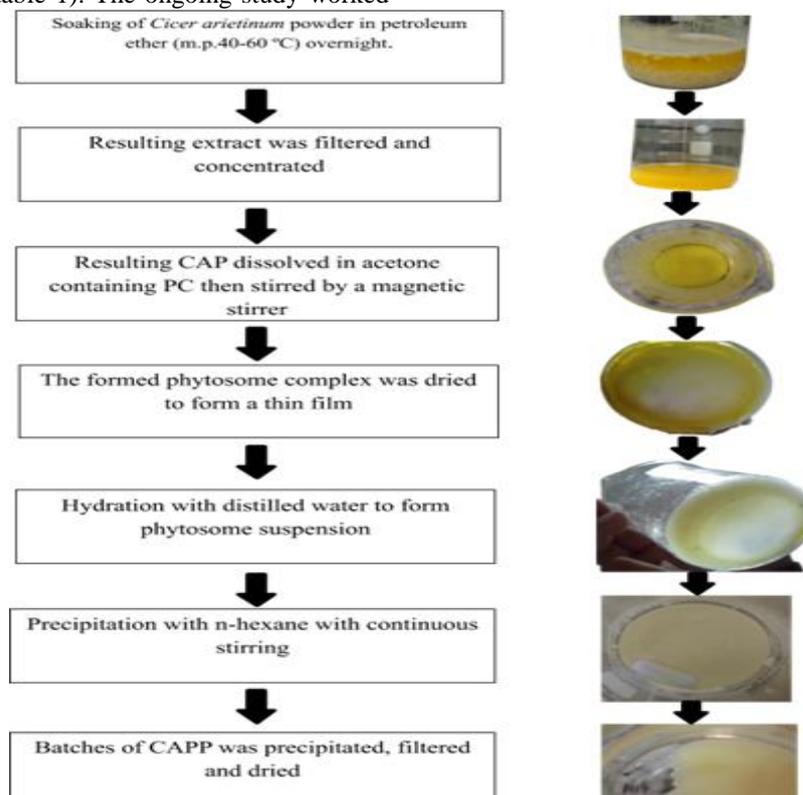


**Figure 2:** Representative diagram shows the preparation of CAPP

### Experimental Animals and design

Adult male albino rats (*Rattus norvegicus*, 150–170 g) used as animal model. Rats were purchased from the animal house of the National Research Center (NRC), Egypt and housed for one week before the beginning of the main experiment in the animal room of the Faculty of Science, Cairo University, Egypt to adapt the new animal room. The caring of animals obeys the recommendations for the proper care and use of laboratory animals of Institutional Animal Care and Use Committee (IACUC) (CUFS/F/PHY/16/15) of the Faculty of Science, Cairo University, Egypt.

## 3. Experimental Design

Forty-eight rats were divided randomly into eight groups (6 rats/ group) as follows:

Normal group: Rats received normal diet and administered distilled water orally for 45 days.
PC group: Rats received normal diet and administered distilled water for 15 days, then administered PC (500 mg/kg body weight, p.o) for another 30 days.
CAP group: Rats received normal diet and administered distilled water for 15 days, then administered CAP (500 mg/kg body weight, p.o) for another 30 days.
CAPP group: Rats received normal diet and administered distilled water for 15 days, then administered CAPP (500 mg/kg body weight, p.o) for another 30 days.
NAFLD group: Rats received high fat diet (HFD) and injected tamoxafene (TAM) (200 mg/kg body weight) intraperitoneally for 15 consecutive days, then administered distilled water for another 30 days.
NAFLD + PC group: Rats received HFD and injected with tamoxafene for 15 days, then administered PC for an additional 30 consecutive days.
NAFLD + CAP group: Rats received HFD and injected with tamoxafene for 15 days, then administered CAP for an additional 30 consecutive days.
NAFLD + CAPP group: Rats received HFD and injected with tamoxafene for 15 days, then administered CAPP for an additional 30 consecutive days.

Twenty-four hour after the last treatment, rats were euthanized using sodium pentobarbital (50 mg/kg body weight) after being fasted overnight. Blood samples were withdrawn by the heart puncture in centrifuge tube and centrifuged at 3000 rpm for 20 min. The serum obtained was stored at -20 $^o$C until used for the determination of different oxidative/antioxidative markers.

## 4. Determination of some Oxidative/ Antioxidative Levels

### Determination of serum lipid peroxidation (s-LPO)

Serum lipid peroxidation (s-LPO) concentration was estimated colorimetrically according to Buege and Aust (1978). This method depends on the generation of thiobarbituric acid reactive substance (TBARS) that produced by the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA) in acidic medium at 95°C. Briefly, 200 µL serum was added to a 1.0 mL TBA and kept for 30 minutes in a boiling water bath, then cooled and measured at 535 nm.

### Determination of serum glutathione reduced (s-GSH)

Serum GSH (s-GSH) estimation depends on the reduction of DTNB [5, 5`dithiobis (2-nitrobenzoic acid)] (Elman's component) with GSH. A 100 µL serum was added to 500 µL of trichloroacetic acid (TCA), then centrifuged at 3000 rpm for 15 minutes. The obtained supernatant was added to 1.0 mL Tris buffer (pH 8.5) and 100 µL DTNB. The whole mixture was allowed to stand at room temperature for 10 minutes and then the absorbance of the sample was read at 405 nm against blank reagent (Beutler et al., 1963).

### Determination of serum superoxide dismutase (s-SOD)

Serum superoxide dismutase (s-SOD) was determined by measuring the ability of enzyme to inhibit the phenazine methosulphate (PMS)-mediated reduction of nitroblue tetrazolium (NBT) dye. A 100 µL serum was placed into a test tube containing a 1.0 mL of working reagent (phosphate buffer pH 8.5, nitroblue tetrazolium, and NADH with ratio of 10:1:1). The reaction was initiated by the addition of 100 µL PMS. This procedure determines all forms of SOD (Cu/Zn SOD, Mn SOD, and Fe SOD) (Nishikimi et al., 1972).

### Determination of serum glutathione peroxidase (s-GPx)

Serum glutathione peroxidase (s-GPx) activity was measured according to the UV method described by Paglia and Valentine (1967). A 10 µL serum sample was added to a mixture of 1.0 mL buffer (pH 7.0), 100 µL nicotinamide adenine dinucleotide phosphate (NADPH) and 100 µL $H_2O_2$. The decrease in absorbance was recorded over 5 minutes at 340 nm against deionized water.

### Determination of serum glutathione reductase (s-GRD)

Serum glutathione reductase (s-GRD) activity was estimated independence of Goldberg and Spooner (1983) method. Where, a50 µl of serum was added to a mixture of 1000 µl of R1 (Potassium phosphate buffer (pH 7.5) and EDTA), 100 µl of GSSG and 100 µl of nicotinamide adenine dinucleotide phosphate (NADPH). The decrease in absorbance was recorded over 5 minutes at 340 nm against air.

## 5. Results

### Effect of CAP and CAPP on hepatosteatosis-induced oxidative damage

A non-statistical alteration was recorded between each of PC, CAP, CAPP rats and normal group concerning the s-LPO, s-GSH, s-SOD, s-GPx, s-GRD concentrations. Nonalcoholic fatty liver disease (NAFLD) stimulated oxidative stress, this proved by significant increment (P< 0.05) in s-LPO level comparison to normal group. Treatment with PC, CAP, and CAPP after induction of NAFLD significantly lessened (P< 0.05) the increased s-LPO concentration, as compared to fatty liver group. On the other hand, significant decline (P< 0.05) in s-GSH, s-SOD, s-GPx and s-GRD concentrations was observed in NAFLD group compared with the normal group. Administration of PC, CAP, and CAPP elevated significantly (P<0.05) the activity of the estimated antioxidative molecules in serum in

comparison with hepatosteatotic rats (Table 2). Also, current data shown that CAPP recovers NAFLD more than PC and CAP. This evidenced by an improvement of the most important serum oxidative stress markers (LPO, GSH, GPx, and GRD) by percentage of -41.29 %, 65.63%, 22.64%, and 46.16%, respectively (Figs. 3-7). However, in the case of SOD the PC administration shows better results by 31.1%.
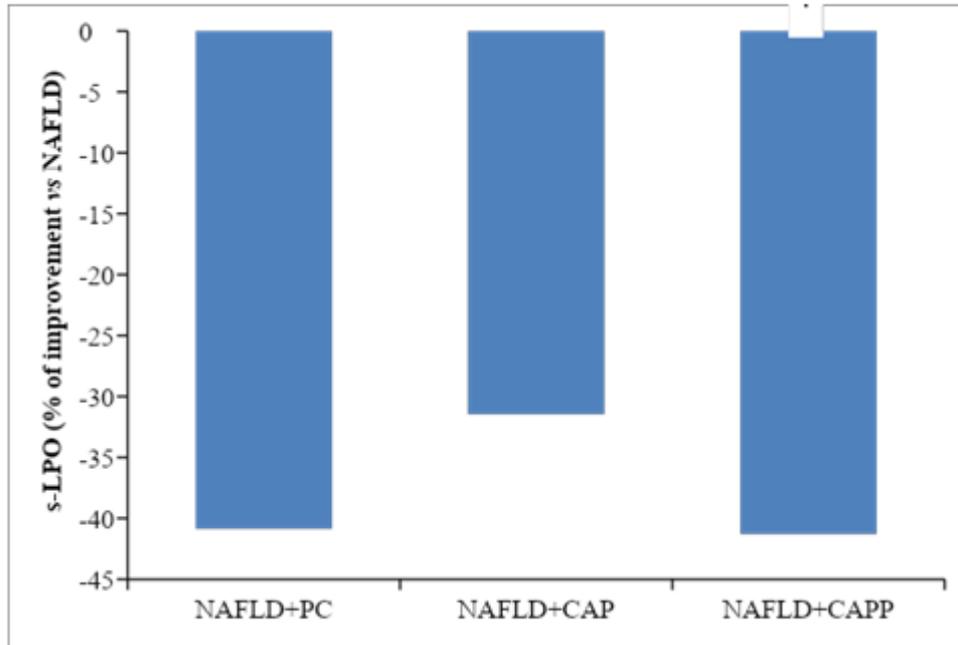


**Figure 3:** Percentage of improvement in serum LPO level of NAFLD rats post-treated with PC, CAP and CAPP. NAFLD: Non-alcoholic fatty liver disease, PC: Phosphatidyle choline, CAP: *Cicer arietinum* polyunsaturated fatty acids, CAPP: *Cicer arietinum* polyunsaturated fatty acids phytosome. *Represent the most effective treatment.
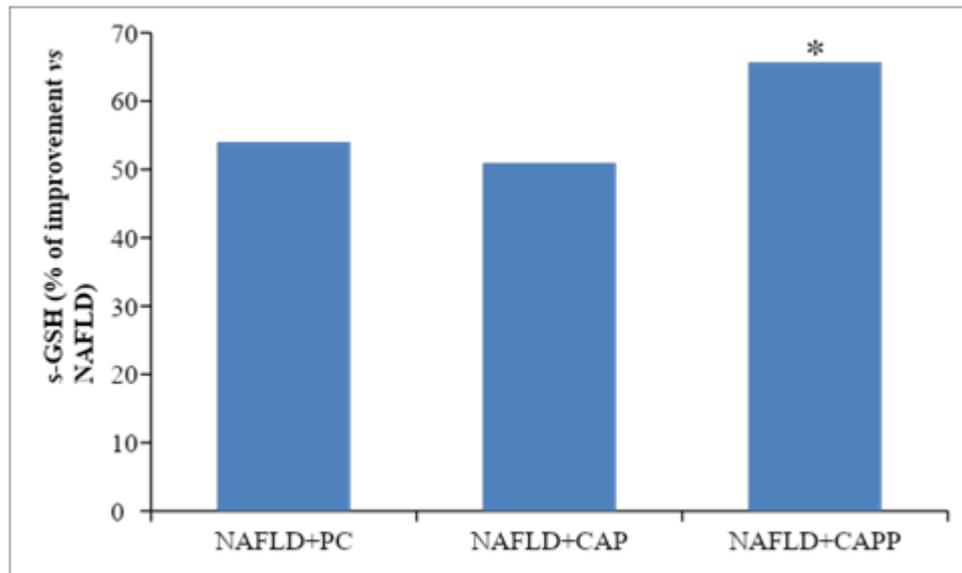


**Figure 4:** Percentage of improvement in serum GSH level of NAFLD rats post-treated with PC, CAP and CAPP. NAFLD: Non-alcoholic fatty liver disease, PC: Phosphatidyle choline, CAP: Cicer arietinum polyunsaturated fatty acids, CAPP: Cicer arietinum polyunsaturated fatty acids phytosome. *Represent the most effective treatment.
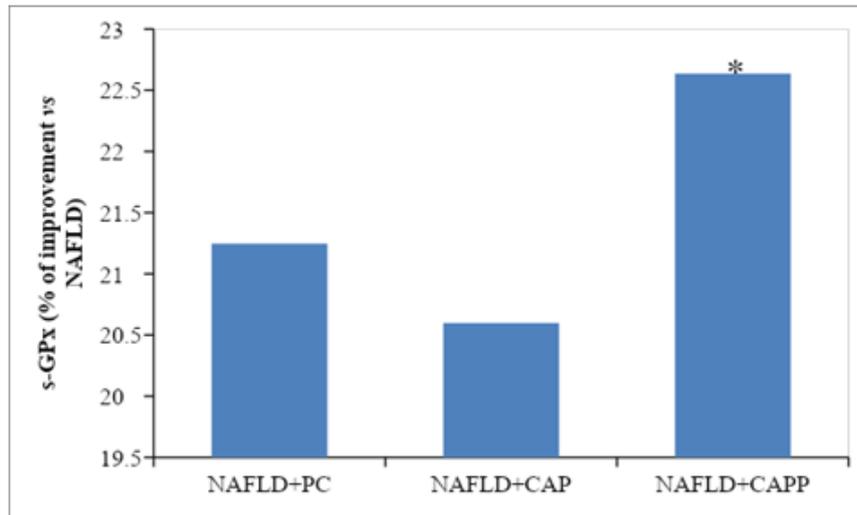
**Figure 5:** Percentage of improvement in serum GPX level of NAFLD rats post-treated with PC, CAP and CAPP. NAFLD: Non-alcoholic fatty liver disease, PC: Phosphatidyle choline, CAP: Cicer arietinum polyunsaturated fatty acids, CAPP: Cicer arietinum polyunsaturated fatty acids phytosome. *Represent the most effective treatment.
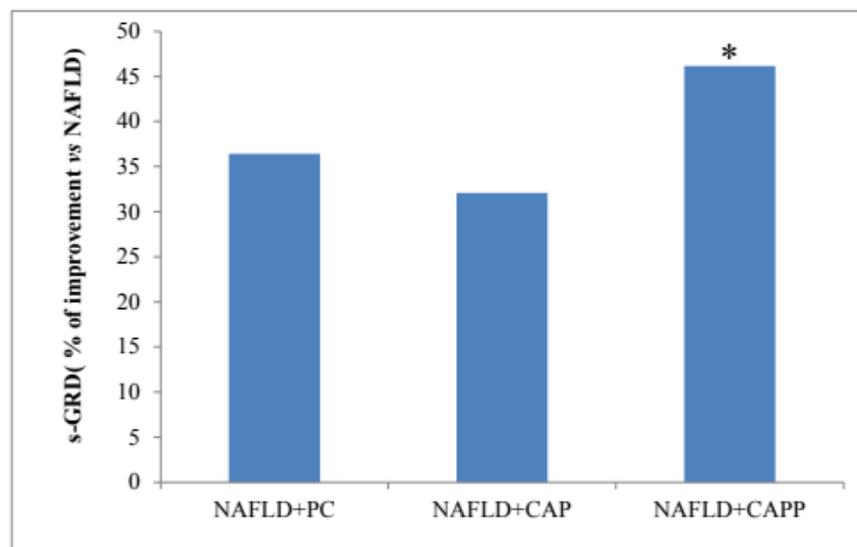


**Figure 6:** Percentage of improvement in serum GR level of NAFLD rats post-treated with PC, CAP and CAPP. NAFLD: Non-alcoholic fatty liver disease, PC: Phosphatidyle choline, CAP: Cicer arietinum polyunsaturated fatty acids, CAPP: Cicer arietinum polyunsaturated fatty acids phytosome.*Represent the most effective treatment
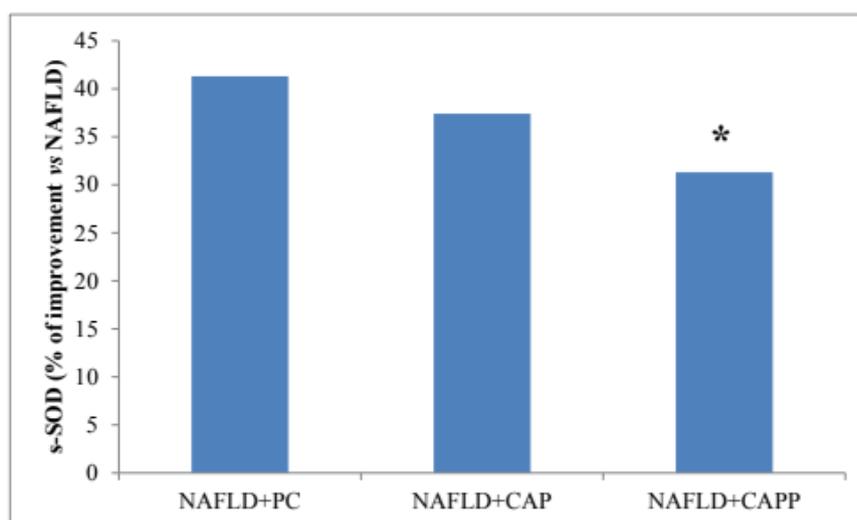


**Figure 7:** Percentage of improvement in serum SOD level of NAFLD rats post-treated with PC, CAP and CAPP. NAFLD: Non-alcoholic fatty liver disease, PC: Phosphatidyle choline, CAP: *Cicer arietinum* polyunsaturated fatty acids, CAPP: *Cicer arietinum* polyunsaturated fatty acids phytosome.*Represent the most effective treatment.

**Table 2:** Effect of CAP and CAPP on oxidative/antioxidative markers

| Parameters Groups | s-LPO (nmol/ml) | s-GSH (nmol/ml) | s-SOD (U/L) | s-GPx (U/mL) | s-GRD (U/L) |
|---|---|---|---|---|---|
| Control | 279.91± 9.18[a] | 178.35± 2.40[a] | 162.30± 6.62[ab] | 7966.16± 352.33[a] | 1427.14± 29.50[ab] |
| PC | 192.17± 8.85[b] | 154.15± 10.21[b] | 156.52± 4.02[a] | 6142.40± 452.96[b] | 1330.23± 35.32[ac] |
| CAP | 176.32± 7.49[b] | 169.15± 5.94[ab] | 174.71± 2.09[b] | 6526.61± 257.32[b] | 1418.15± 14.13[ab] |
| CAPP | 189.14± 10.14[b] | 173.89± 9.55[a] | 154.21± 3.61[a] | 8481.67± 283.82[a] | 1454.19± 34.50[b] |
| NAFLD | 358.57± 8.77[c] | 111.31± 2.96[c] | 113.81± 3.05[c] | 6265.28± 119.27[b] | 972.12± 39.11[d] |
| NAFLD+ PC | 212.15± 7.94[bd] | 171.39± 4.39[b] | 160.80± 5.54[a] | 7596.54± 291.56[a] | 1284.07± 33.62[c] |
| NAFLD+ CAP | 245.79± 3.66[d] | 167.95± 3.44[ab] | 156.36± 4.01[a] | 7555.75± 133.75[a] | 1326.27± 54.56[ac] |
| NAFLD+ CAPP | 210.53± 3.45[bd] | 184.35± 3.10[a] | 149.43± 5.76[a] | 7683.43± 369.65[a] | 1420.84± 10.36[ab] |

Values are expressed as mean ± SEM (n=6), different bold superscript letters are significantly different (P < 0.05), CAP: *Cicer arietinum* PUFA-rich, CAPP: *Cicer arietinum* PUFA-rich phytosome, NAFLD: Non-alcoholic fatty liver disease, PC: phosphatidylcholine.

## 6. Discussion

Non-alcoholic fatty liver disease (NAFLD) is considered the most common cause of chronic liver disease worldwide. NAFLD that results by overeating of lipid-riched food or *de novo* hepatic synthesis of fatty acids increased hepatic influx of lipids (Jou et al., 2008; Tiniakos et al., 2010; and Vanni et al., 2010). Thereby, the present study focused on the alleviation of NAFLD by using the yellow split-desi chickpea (Chana Dal) phytosome to enhance the absorption of the proactive agents of Chana Dal.

The present study revealed that oxidative injury in NAFLD rats was established as evidenced by increased serum LPO level, decreased GSH, SOD, GPx, and GRD levels. The current results are inconsistent with Hassanein and El-Amir (2018). The present study explained the increased oxidative damage in NAFLD group due to its injection with tamoxifen. TAM induces hepatosteatosis through impairment of mitochondrial fatty acid oxidation (FAO) as a primary cause of lipid accumulation in the liver (Cardoso et al., 2001; Letteron et al., 2007 & Zhao et al., 2014). Thereby, it causes inhibition of β-oxidation of free fatty acids, lipoprotein secretion from the liver, and hepatic triacylglyceride secretion leading to lipid accumulation in liver (Letteron et al., 2003; Letteron et al., 2007 and Zhao et al., 2014). Further, tamoxifen produces overproduction of oxidative stress during their metabolisms in the liver Lipid peroxidation of NAFLD group provoked by free radicals attack the biological structures causing severe hepatosteatosis. Indeed, measurement of LPO has been used as a sensitive assay for oxidative damages of lipids (Grotto et al., 2009). Additionally, oxidative damage is produced in NAFLD rats due to the significant decrease of the GSH content. GSH is a major naturally occurring non-protein thiol antioxidant in living organisms, which prevents oxidative damage by elimination of reactive intermediates and helps detoxification by conjugating with chemicals (Lushchak, 2011). Again, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for vital antioxidant enzymes (Zoidis et al., 2018). Liver injury has been observed when GSH stores are markedly depleted. The decreased GSH in the present study may be due to their utilization to minimize the overproduction of oxidative threats.

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The antioxidant enzymes, including SOD, GPx and GRD are a mutually supportive team of defense against ROS. Their significant decrease in NAFLD rats exaggerates the oxidative stress. As the superoxide dismutase (SOD) is a metalloprotein that catalyzes the dismutation of superoxide radicals (Younus, 2018). In this respect, SOD accelerates the dismutation of $H_2O_2$, which prevents the further generation of free radicals (Liu et al., 2010). The present study interprets the decreased SOD level in the NAFLD group to an excessive formation of superoxide anions. GPx is a selenoenzyme, two third of which is present in the cytosol and one third in the mitochondria. GPx plays a major role in the reduction of hydrogen peroxide and hydroperoxide to nontoxic products (Thirumalai et al., 2011). These enzymes work together to scavenge oxygen species. Glutathione reductase (GRD) is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells (Couto et al., 2016). GRD determines the most suitable conditions for redox control within a cell by recycling oxidized glutathione. Where it catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH). The latter plays a key role in maintaining proper function to resisting oxidative stress in human cells by maintaining the reducing environment of the cell (Deponte, 2013). It can act as a scavenger of hydroxyl radicals, singlet oxygen, and various electrophiles. The ratio of GSSG/GSH present in the cell is a key factor in properly maintaining the oxidative balance of the cell that is critical for the cell to maintain high levels of the GSH and a low level of the GSSG. This narrow balance is maintained by glutathione reductase, which catalyzes the reduction of GSSG to GSH (Deponte, 2013). The present results indicated that HFD and TAM significantly decreased serum SOD, GPx, and GRD activities. These results were in line with previous studies which have shown that NAFLD generates lipid peroxidation and fluctuate the antioxidant status in rats (Aly and Kleiner, 2011). The decline in the antioxidant enzymes can be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in an inactivation of the $H_2O_2$ scavenging enzymes. Additionally, the present study suggests that the marked reduction in GPx activity, leading to reduced availability of substrate for GRD, thereby decreasing the activity of GRD. Further, may be due to the decreased synthesis of enzymes or oxidative deactivation of enzyme protein.

The overproduction of free radicals can lead to hepatosteatosis. As the cell membrane is a critical barrier for free radicals. Free radicals need to pass through this barrier in order to interact with intracellular components. The increasing LPO and decreasing antioxidant molecules

resulted in damaging the liver tissue and increase the severity of hepatosteatosis induced by HFD and tamoxifen (Chang et al., 2018). Free radicals initiate lipid peroxidation by eliminating the hydrogen atom from alpha-methylene groups of polyunsaturated fatty acids in the cell membrane (li et al., 2015). At the end of the process, polyunsaturated fatty acids are hydrolyzed into biologically active compounds. The most important of these compounds is MDA which reflects lipid peroxidation in the body.

Treatment of hepatosteatosis has been often expensive, especially in developing countries that eventually leading to liver cirrhosis in the population. Thereby, treatment using the ethnobotanical approach has gained popularity as an alternative cost effective approach (Saleem et al., 2010). The natural medicine has been widely applied. It serves as an alternative complementary medicine, probably due to the presence of the complete pharmacopeia of the herbs and other agents (Abd. Rahim et al., 2017). The present study recorded that CAP and CAPP decreased s-LPO significantly either directly or through increasing GSH that scavenging the free radicals. As well as, there was a significant increase in the s-SOD, s-GPx, and s-GRD in NAFLD rats treated with CAP or CAPP. GSH together with the antioxidant enzymes amplify the scavenging of ROS efficiently. They may protect cellular constituents from the damaging effects of peroxides formed during metabolism and other ROS. The current results evidenced that CAPP is more efficient than CAP in restoring the antioxidant status to near the normal values. Administration of CAP or CAPP to NAFLD restores the activity of GRD, this may accelerate the utilization of GSSG to form GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH. Further, the proactive agents of CAP become easily absorbed when administered in the form of phytosome (CAPP). Moreover, CAPP enriched with PUFA which may protect the SH group of GSH from the reactive radicals (Abdou and Hassan, 2014). The antioxidant effect of PUFA through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously by Pauwels and Kostkiewicz (2008). So, CAPP may have a greater ability to stabilize the ROS by reacting with them and getting oxidized in turn to more stability. In consonance with the present study, Naik and Panda (2008) noticed the hepatoprotective activity of ginkgoselect phytosome (GBP) and its effect on the antioxidant status in rats. Tung et al. (2017) publicized that oral administration of 200 mg/kg of curcumin phytosome, four or seven days produced a significant reduction in the lipid peroxidation level and elevated enzymatic antioxidant activities.

## 7. Conclusion

The present study evaluates the effect of yellow-split *C. arietinum*-enriched PUFA (CAP) and its phytosome form (CAPP) against the hepatosteatosis induced by HFD and TAM from the side of oxidative stress. This study revealed that CAPP is more efficient than CAP as it has beneficial responses to significantly attenuate the serum oxidative damage of hepatosteatosis. This finding highlights the importance to study the more detailed mechanistic effect of CAPP in future study.

## Reference

[1] Abd Rahim, E. N. S., Ismail, A., Omar, M. N., Rahmat, U. N. and Ahmad, W.A.N.2017. GC-MS Analysis of Phytochemical Compounds in Syzygium polyanthum Leaves Extracted using Ultrasound-Assisted Method. Pharmacogn J. 10(1), 110-119.

[2] Abdou, H. M. and Hassan, M.A. 2014. Protective Role of Omega-3 Polyunsaturated Fatty Acid against Lead Acetate-Induced Toxicity in Liver and Kidney of Female Rats. BioMed Research International. 5(3), 1-11.

[3] Aguirre, L., Portillo, M. P., Hijona, E. and Bujanda, L. 2014. Effects of resveratrol and other polyphenols in hepatic steatosis. World J Gastroenterol. 20(23), 7366-7380.

[4] Aly, F.Z. and Kleiner, D. 2011. update on fatty liver disease and steatohepatitis. National institutes of health. 18(4), 294–300.

[5] Amit, P., Tanwar, Y.S., Rakesh, S. and Poojan, P. 2013. Phytosome: Phytolipid Drug Dilivery System for Improving Bioavailability of Herbal Drug. Journal of pharmaceutical science and bioscientific research. 3(2), 51-57.

[6] Beutler , E., Duron, O. and Kelly, B. M. 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med, 61. 882– 888.

[7] Bhattacharya, S. and Ghosh, A. 2008. Phytosomes: the Emerging Technology for Enhancement of Bioavailability of Botanicals and Nutraceuticals. The Internet Journal of Aesthetic and Antiaging Medicine. 2(1), 1-8.

[8] Buege, J. A., and Aust, S. D. 1978. Microsomal lipid peroxidation. Method Enzymol, 52, 302-310.

[9] Cardoso, C. M. P., Custódio, J. P. A., Almeida, L. M. and Moreno, A. J. M. 2001. Mechanisms of the Deleterious Effects of Tamoxifen on Mitochondrial Respiration Rate and Phosphorylation Efficiency. Toxicology and Applied Pharmacology, 176(3), 145-52.

[10] Chang, H. T., Pan, H. J. and Lee, C.H. 2018. Prevention of Tamoxifen-related Nonalcoholic Fatty Liver Disease in Breast Cancer Patients. Clinical Breast Cancer, 18(4), e677-e685.

[11] Chena, Z., Tiana, R., Shea, Z., Cai, J. and Li, H. 2020. Corrigendum to "Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. Free Radical Biology and Medicine, 152 (2020), 116–141.

[12] Cole, L. K., Jacobs, R. L. and Vance, D. E. 2010. Tamoxifen Induces Triacylglycerol Accumulation in the Mouse Liver by Activation of Fatty Acid Synthesis. Steatohepatitis/metabplic liver disease, 52(4), 1258- 1265.

[13] Couto, N., Wood, J. and Barber, J. 2016. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radical Biology and Medicine. 95, 27–42.

[14] Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A. and Larson, S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal, 9(10), 1-12.

[15] Deponte, M. 2013. Glutathione catalysis and the reaction mechanisms ofglutathione-dependent

enzymes. Biochimica et Biophysica Acta. 1830, 3217–3266.

[16] Divella, R., Mazzocca, A., Daniele, A., Sabbà, C. and Paradiso, A. 2019. Obesity, Nonalcoholic Fatty Liver Disease and Adipocytokines Network in Promotion of Cancer. International Journal of Biological Sciences, 15(3), 610-616.

[17] Gandhi, A., Dutta, A., Pal, A. and Bakshi, P. 2012. Recent Trends of Phytosomes for Delivering Herbal Extract with Improved Bioavailability. Journal of Pharmacognosy and Phytochemistry, 1 (4), 6-14.

[18] Grotto, D., Santa, S., Maria, Valentini,J., Paniz,C. and Schmitt,G. 2009. Importance of the lipid peroxidation biomarkers and methodological aspects FOR malondialdehyde quantification. Quim. Nova, 32(1), 169-174.

[19] Hassaneina, K. M.A. and El-Amira, Y. O. 2018, Ameliorative effects of thymoquinone on titanium dioxide nanoparticles induced acute toxicity in rats. International Journal of Veterinary Science and Medicine, 6, 16-21.

[20] Helal, E. G. E., Abd El-Wahab, S. M., Sharaf, A. M. M. and Zedan, G. A. 2011. Effect of Cichorium intybus L. on fatty liver induced by oxytetracycline in albino rats. The Egyptian Journal of Hospital Medicine, 45, 522 – 535.

[21] Janevski, M., Antonas, K. N., Sullivan-Gunn, M. J., McGlynn, M. A. and Lewandowski, P. A. 2011. The effect of cocoa supplementation on hepatic steatosis, reactive oxygen species and LFABP in a rat model of NASH. Comparative hepatology, 10(10), 1-13.

[22] Johnston, S. J.and Cheung, K. L. 2018 Endocrine Therapy for Breast Cancer: A Model of Hormonal Manipulation. Oncol Ther, 6, 141–156.

[23] Jou, J., Choi, S. and Diehl, A. 2008. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis. 28, 370–9.

[24] Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N. 2012. Nutritional uality and health benefits of chickpea (Cicer arietinum L.). British Journal of Nutrition, 108 (S1), S11-S26.

[25] Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N. 2012. Nutritional quality and health benefits of chickpea (Cicer arietinum L.). British Journal of Nutrition, 108, S11–S26.

[26] Kawano, Y. and Cohen, D. E. 2013. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. J Gastroenterol, 48, 434–441.

[27] Lee, J. E., Lee, Y. J., Chung, S. Y., Cho, H. W., Park, B. J., Jung, D.H. 2018. Severity of nonalcoholic fatty liver disease is associated with subclinical cerebrocardiovascular atherosclerosis risk in Korean men. PLoS ONE, 13(3), 1-11.

[28] Letteron, P., Sutton, A., Mansouri, A., Fromenty, B., Pessayre, D. 2003. Inhibition of microsomal triglyceride transfer protein: another mechanism for drug-induced steatosis in mice. Hepatology, 38, 133–140.

[29] Lettéron, P., Larosche, I., Fromenty, B., Vadrot, N., Toby, A.A., Feldmann, G., Pessayre, D. and Mansouri, A. 2007. Tamoxifen Inhibits Topoisomerases, Depletes Mitochondrial DNA, and Triggers Steatosis in Mouse Liver. Journal of Pharmacology and Experimental Therapeutics, 321(2), 526-535.

[30] Li, S., Tan, H., Wang, N., Zhang, Z., Lao, L., Wong, C. and Feng, Y. 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International journal of molecular science. 16, 26087–26124.

[31] Liu, J., Li, M. and Zhao, L. 2010. Multi-mechanisms are involved in reactive oxygen species regulation of mTORC1 signaling. Cell Signal. 22, 1469–1476.

[32] Lushchak, V. I. 2011. Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions. Journal of Amino Acids, 2012, 1-26.

[33] Mahmoud, H. A., Yousry, W. A., Saleh, S.A., El Badry M., Hussein A., Mostafa Hassan Ali, M. H. and El-Hariri, H. M. 2016. Renal resistive index in non-alcoholic fatty liver disease as an indicator of early renal affection. Egyptian Liver Journal, 10(6), 1-8.

[34] Mitra, S., De, A. and Chowdhury, A. 2020. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. Translational Gastroenterology and Hepatology, 5(16), 1-17.

[35] Naik, S. R. and Panda, V. S. 2008. Hepatoprotective effect of Ginkgoselect Phytosome in rifampicin induced liver injury in rats: evidence of antioxidant activity. Fitoterapia, 79 (6), 439-445.

[36] Nishikimi, M., Appaji, N. and Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen.Biochem Biophys Res Commun, 46(2), 849-854.

[37] Paglia, D. E. and Valentine, W. N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70(1), 158-169.

[38] Pauwels, E. K. J and Kostkiewicz, M. 2008. Fatty acid facts, part III: cardiovascular disease, or, a fish diet is not fishy. Drug News and Perspectives, 21(10), 552–561.

[39] Pydyn, N., Miękus, K., Jura, J. and Kotlinowski, J. 2020. New therapeutic strategies in nonalcoholic fatty liver disease: a focus on promising drugs for nonalcoholic steatohepatitis. Pharmacological Reports, 72, 1–12.

[40] Quijano, C., Trujillo, M., Castro, L. and Trostchansky, A. 2015. Interplay between oxidant species and energy metabolism. Redox Biology, 8, 28–42.

[41] Rahman, H. S., Othman, H. H., Hammadi, N. I., Yeap, S. K., Amin, K. M., Samad, N. A. and Alitheen, N. B. 2020. Novel Drug Delivery Systems for Loading of Natural Plant Extracts and Their Biomedical Applications. International Journal of Nanomedicine, 15, 439–483.

[42] Salama, M. A., Owon, M. A., Osman, M. F., Esmail, A,. I. and Matthäus, B. 2018. Characterization of Egyptian Moringa oleifera Lipids (Whole Seeds and Kernels). J. Food and Dairy Sci., 9 (12), 379 – 384.

[43] Saleem, M. T. S., Chetty, C. M., Ramkanth, S., Rajan. V. S. T., Kumar, K. M. and Gauthaman, K. 2010. Hepatoprotective herbs: A review. International journal of researches of pharmaceutical sciences. 1, 1-5.

[44] Sánchez, N. M., Rodríguez, A. V., Castillo, C. C., Barajas, A. V., Carmona, J. C., Rodríguez, G. P. and

Valdés, D. Z. 2020. The cellular pathways of liver fibrosis in non-alcoholic Steatohepatitis. Annals of Translational Medicine, 8(6), 1-14.

[45] Takahashi, Y., Soejima, Y. and Fukusato, T. 2012. Animal models of nonalcoholic fatty liver disease/ nonalcoholic steatohepatitis. World J Gastroenterol , 18(19), 2300-2308.

[46] Thirumalai, T., Viviyan, T. S., Elumalai, E. K. and David, E. 2011. Hypolipidaemic and antioxidant effect of Enicostemma littorale Blume. Asian Pacific Journal of Tropical Biomedicine, 1(5), 381-385.

[47] Tiniakos, D. G.,Vos, M. B. and Brunt, E. M. 2010. Nonalcoholic Fatty Liver Disease: Pathology and Pathogenesis. Annual Review of Pathology Mechanisms of Disease, 5(1), 145-71.

[48] Tung, B. T., Hai, N. T. and Son, P. K. 2017. Hepatoprotective effect of Phytosome Curcumin against paracetamol-induced liver toxicity in mice. Brazilian Journal of Pharmaceutical Sciences, 5 (31), 1-13.

[49] Vanni, E., Bugainesi, E., Kotrone, A., Minicis, S. D., Yki-J., H. and Baroni, G. S. 2010. From the metabolic syndrome to NAFLD or vice versa. Digestive and liver diseases, 42(5), 320-330.

[50] Yoshitomi, H., Guo, X., Liu, T. and Gao, M. 2012. Guava leaf extracts alleviate fatty liver via expression of adiponectin receptors in SHRSP.ZLeprfa/Izm rats. Nutrition & Metabolism, 9(13), 1-7.

[51] Younus, H. 2018. Therapeutic potentials of superoxide dismutase. International Journal of Health Sciences, 12(3), 88-93.

[52] Zhao, F., Xie, P., Jiang, J., Zhang, L., An, W. and Zhan Y. 2014. The Effect and Mechanism of Tamoxifen-Induced Hepatocyte Steatosis in Vitro. International Journal of Molecular Sciences, 15, 4019-4030.

[53] Zoidis, E., Seremelis, I., Kontopoulos, N. and Danezis, G. P. 2018. Selenium-Dependent Antioxidant Enzymes:Actions and Properties of Selenoproteins. Antioxidants, 7(66), 1-26.