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RESEARCH ARTICLE

Therapeutic Intervention of *Opuntia Ficus Indica* (L.) Fruit and Seed Powder against CCl_4-Induced Acute Liver Injury in Wistar Rats

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ABSTRACT

The liver is the largest metabolic organ performing metabolic, hematological functions and detoxifying toxins. Liver toxicity is the major outcome of environmental and metabolic toxins. HPLC analysis were conducted on Opuntia Ficus Indica (OFI) seed powder and fruit powder (FP) analyzed the total phenolic, total flavonoid, and quantified the nutritional composition, mineral composition, vitamin E, vitamin C as well the level of antioxidants. The efficacy study was closed labeled and consisted of twenty-eight days. The study was designed to analyze the antioxidant-dependent anti-inflammatory efficacy of *Opuntia Ficus Indica* (OFI) fruit powder (FP) and *Opuntia Ficus Indica* (OFI) seed powder (SP) against carbon tetra chloride (CCl_4) induced acute liver injury in Wistar rats. The first negative control group (n=10) was fed on a standard diet and the second positive control group (n=10) after liver injury through induction of carbon tetrachloride (CCl4) with the normal standard diet. Treatment groups received fruit powder (FP) and seed powder (SP) after induction of CCL4. Baseline parameters including liver functioning test, ALP, ALT and serum bilirubin were analyzed and study results revealed that the liver enzymes such as elevated ALP, ALT, and serum bilirubin were significantly ($P<0.05$) resolved in all treatment groups compared with control positive group. Oxidative stress markers were significantly improved in all treatment groups compared with the control group such as total antioxidant capacity was increased, and total oxidative stress was reduced in the blood serum of all treatment group fruit powder (FP), seed powder (SP) compared to the control group.

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INTRODUCTION

Inflammation is the tissue-repairing mechanism, while inflammatory diseases are considered the onset of many chronic diseases. Drugs are a therapeutic application against the subset of inflammation (Loomba *et al*., 2021). The liver is the vital organ for the metabolic, hematological, and detoxification of toxins (Badawi, 2022). There are two types of hepatotoxicity or liver injury i.e., acute and chronic injury. Acute hepatotoxicity is a hepatocellular injury that lasts for less than a few days to a month and chronic hepatotoxicity is difficult to diagnose with persistence beyond months (Fathy and Mahmoud, 2021). Chemicals causing hepatoxicity are mainly carbon tetrachloride, HCL, sulfuric acid, sodium hydroxide, and gasses particularly methane causing hepatic problems (Chen *et al*., 2021). Analgesics drugs such as Aspirin and Paracetamol are also the major inducer of liver injury that can cause the onset of liver inflammation (Björnsson and Björnsson, 2022).

Not only medicines but chemicals also cause damage to liver tissue and the onset of inflammation triggered by oxidative stress (Roobi *et al*., 2022) by reducing the antioxidants and causing liver toxicity (Yi *et al*., 2021). Hepatotoxicity initiates oxidative stress through activation of reactive oxygen species (ROS), age, lipid peroxidation of hepatocyte films, hepatic irritation alongside pro-inflammatory cytokines inducible, e.g., TNF-α and interleukin-1β (Sun *et al*., 2022). There is an increased level of cytokines such as IL-1, IL-6, TNF- α, and IL-10 which prompt inflammation of liver parenchyma (Tang *et al*., 2021).

Now days, herbal medicines are increased due to better safety, cheap with low side effects in comparison to allopathic medicine which has been found to pose serious side effects (Wang *et al*., 2022). *Opuntia Ficus Indica* (OFI) is also used as a medicinal fruit that belongs to the *Cactaceae* family and fruit has effective antioxidant properties and contains vitamins E and C. Vitamin E and C play a very crucial role in reducing oxidative stress and ceasing IL-6 and tumor necrosis factor-alpha inhibits inflammation and reversing liver enzyme levels (Daniloski *et al*., 2022). Beta-lains in *Opuntia Ficus Indica* fruit can protect endothelial cells from oxidation and promote interleukins to prevent inflammation of liver and liver enzymes (Sutor-Świeży *et al*., 2022; Carreón-Hidalgo *et al*., 2022). Vitamin E and C in prickly pear reduces oxidative stress as well as inflammation (Zielinska-Przyjemska *et al*., 2009).

Increased levels of free radicals and oxidants produced by toxic products in the body are responsible for the synthesis and accumulation of ROS in the cell (Liu *et al*., 2022). These oxidants and free radicals are responsible for the onset of oxidative stress and producing ROS which leads to disrupt cellular processes. Similarly, lipids (Causing lipid peroxidation) and nucleic acids (inducing DNA damage and strand breaks) also produce ROS (Hvidtfeldt *et al*., 2021). ROS includes the superoxide anion (O2−), hydrogen peroxide (H2O2), hydroxyl radical (·OH) and singlet oxygen (1O2) (Kalantari *et al*., 2018). ROS is the hydroxyl radical and can react with almost every tissue directly, thereby causing more effective cellular damage (Tang *et al*., 2021).

MAPK (Mitogen-activated protein kinase) signaling pathways are the main ones responsible for the onset of proinflammatory cytokines and inflammatory responses. Tumor necrosis factor is responsible for the activation of the TNF Receptor (TNFR) and allows TRAF-2, TRAF-3, TRAF-6, and IKK gamma converted to TNFR complex. TRAF-3 inhibits the activation of MEKK1 and TAK1 at the TNFR complex. Degradation of TRAF-3 releases the MEKK1 and TAK1 complexes to activate MKK4/7 and MKK3/6, then phosphorylate JNK and p38, respectively (Majeed *et al.*, 2023). The activation of these transcription factors results in the transcription of genes encoding proinflammatory cytokines (Ali *et al.*, 2023). In the cytoplasm, the translation of proinflammatory gene mRNA is inhibited by KSRP and TTP (Aslam *et al.*, 2022), both binding to and promoting the degradation of mRNA. The p38 directly inhibits KSRP and indirectly inhibits TTP, via MAPKAPK2, to promote inflammation via the stabilization and translation of proinflammatory gene mRNA (Seo *et al*., 2022).

The proposed research is designed to analyze the anti-inflammatory and antioxidant efficacy of red-purple *Opuntia Ficus Indica* (OFI) fruit powder (FP) and seed powder (SP) against CCl_4 (Carbon Tetra-chloride) induced liver toxicity in Wistar rats. Liver enzymes (Alanine transaminase (ALT), Alkaline phosphatase (ALP), serum bilirubin, antioxidant activity such as superoxide dismutase, Catalase, glutathione peroxidase, and anti-inflammatory markers; interleukins e.g., IL-6, IL-1β, and TNF-α were analyzed to measure the antioxidant and anti-inflammatory efficacy of fruit pulp and seed powder against CCL4 induced liver toxicity.

MATERIALS AND METHODS

Sample preparation of prickly pear: *Opuntia Ficus Indica* (OFI) was procured from the local market of Faisalabad and authenticated by a taxonomist of the Department of Botany, UAF declared Herbarium number; 197-21-01. Fruit was washed; all dirt and spines were removed from the skin. The fruit was cut into small pieces, mixed well into the sonication process in a sonicator, and placed in Harvest Saver Commercial Dehydrator (R-5A, Eugene, OR, USA) at 55°C for 48 hours (Sanchez *et al*., 2006). In the following process, dried fruit was ground into powder, fruit pulp (FP) seed powders (SP) were placed into glass jars and stored at room temperature.

Proximate analysis: Crude protein, fiber, fat, moisture, and ash contents of *O. ficus-indica* fruit and seed powder were analyzed by process of AOAC (2012) Method No. 984-13, Method No. 978-10, Method No. 934-01 and Method No. 942-05 respectively.

Mineral analysis: Digested samples of dried OFI fruit powder (FP) and seed powder (SP) samples were used for the detection of minerals like Na, Ca, and K by using Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge, UK) Method no 956.01. The Mg and Fe were determined (Hitachi Polarized Zeeman AAS, Z-8200, Japan) through a spectrophotometer following the standard procedures of AOAC (2012) Method no 975.03 (b) and 991.11.

Quantification of vitamin C: High-Performance Liquid Chromatography was used for quantification of vitamin C from FP and SP by following the method of Nazareno *et al*., (2007).

β-carotene and vitamin E assessment: The (~2 g) finely grounded OFI-FP and OFI-SP extraction solution was prepared after the addition of ethanol, acetone, and hexane. Progressively, the solution was stirred for 40 minutes on a magnetic hot plate and 10 ml water was added for separation of layers. The upper layer was separated, and residues were further mixed with a methanol/acetonitrile solution. Ultimately, samples were filtered and 20 µl were injected for HPLC analysis. The system consists of a C-18 column, 25 cm × 4.6 mm, and a 5 µm chromatographic separation column. The absorbance of beta carotene was measured at 475nm according to the procedure of Siriamornpuna *et al*., (2012), and vitamin E was measured at 295nm according to Ramadan and Mörsel, (2003).

Phenolics and flavonoids by HPLC: Extraction was carried out with the help of an ultrasonic water bath by the homogenization of 1g samples with 5 ml methanol: 95ml water solution following centrifugation at 4000 rpm at 4ºC, 1% formic in distilled water was mixed with a solvent (1% formic acid in methanol), the 50% separate layer was achieved within 10 minutes and 70% within 15 min. 20µL was injected into HPLC and wavelength of phenolics and flavonoids was checked at 280 nm and 370 nm respectively (García-Cayuela *et al*., 2019).

Total betalains; betaxanthins and beta-cyanins contents: Total betalains, betaxanthins, and beta-cyanins in the extracts of OFI-FP and OFI-SP were carried out by using different buffers, and analysis was done through a spectrophotometer. A 5-gram dried sample was diluted in a phosphate buffer of pH 8 (Stintzing *et al*., 2005) and an acetate buffer of pH 6. Amount of beta-lain content (BC) mg/L was calculated using equation of Stintzing *et al*. (2003). The supernatant was further used for absorbance determination of betaxanthins and betacyanin through a spectrophotometer at 535nm and 482nm respectively (Ravichandran *et al*., 2013).

Selection of experimental rats for bio-efficacy trials: For the bio-efficacy trial, adult healthy Wistar rats (40) approximately weighing 190–250 grams were placed in the animal room at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad. D.No.3477/ORIC Bioethical permission was taken from the "bioethics committee" of the University of Agriculture, Faisalabad, Pakistan. The rats were kept in different cages at constant room temperature (22 ± 2°C) and relative humidity (55% ± 5%). Moreover, all rats were fed on a specific laboratory diet and particular drinking water.

Experimental design for bio-efficacy: Each experimental group consists of 10 Wistar rats, further divided into four groups: group-1, G_1= negative control, group-2, G_2= positive control (CCl4 induced liver toxicity), group-3, G_3= FP fruit powder (1000mg/kg/ b.w) with CCl_4 induced liver toxicity and group-4, G_4= SP seed powder (1000mg/kg / b.w) with induced CCl_4 toxicity along with control diet as prescribed (Table 1) (González-Ponce *et al*., 2016; Bisson *et al.*, 2010).

Table 1: Treatment plans of study group for bio-efficacy intervention trial

Group	Dose
G_0	Normal feed + no induction of CCL_4
G_1	Normal feed + CCL_4 induced toxicity
G_2	Normal feed +CCL_4 induced toxicity + OFI-FP
G_3	Normal feed +CCL_4 induced toxicity + OFI-SP

OFI-FP = *Opuntia Ficus Indica* - fruit powder
OFI-SP= *Opuntia Ficus Indica* - seed powder

***Liver enzyme activity in blood serum*:** Alanine transaminase level in serum was determined by the colorimetric method (El-Said *et al*., 2011). The determination of serum bilirubin is based on Van Den Bergh reaction (Jada *et al*., 2021). ALP alkaline phosphatase activity in the serum of rats was analyzed using (IFFC-DGKCh) regent by kinetic rate method (Hata *et al*., 2021).

***Oxidative Stress Markers in Blood Serum*:** Total oxidative stress and total antioxidant capacity were measured in blood serum. It was measured in mmol Trolox Equiv. /L (Franco-Martinez *et al*., 2016).

***Total oxidant status*:** A blood serum sample was used to calibrate with hydrogen peroxide and findings were expressed in terms of micromolar hydrogen peroxide equivalent per liter (H_2O_2 µmol/L) to analyze the Total oxidant status. (Aliosmanoglu *et al*., 2018).

***Determination of Antioxidant Activity and Lipid Peroxidation in Serum*:** Superoxidase activity in the serum of rat's blood was assayed spectrophotometrically. The SOD activity was calculated in Units / mL for serum blood (Abdel-Daim *et al*., 2020; Nishikimi *et al*., 1972). Catalase activity in blood serum was measured and the activity of catalase was expressed in Units/mL serum for serum samples (Aebi, 1984; Ammar *et al*., 2018). GPx in tissue was determined and glutathione level was expressed as nanomoles/mg of protein (Tatli *et al*., 2000; Jollow *et al*., 1974).

***Inflammatory biomarkers*:** Serum samples were used to measure the levels of TNF-α, IL-β1, and IL-6 in the supernatants. Inflammatory biomarkers were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Ecoline, Merck Germany) according to the manufacturer's instructions provided by the marketer user (Antunes-Ricardo *et al*., 2015; Aristatile *et al*., 2013).

***Histopathology of liver tissue*:** Histopathological examination of liver tissues was done for all experiment groups following the method under (MCX 100, Micros Austria) (Sheha *et al*., 2018; Kafle *et al*., 2018).

Statistical analysis: GraphPad Prism® 8.1 statistical software was used for statistical analysis. All estimations were presented as mean ± SE and collected data were analyzed through ANOVA with a significance level of $P<0.05$ (Montgomery, 2008).

RESULTS

Nutritional composition: Nutritional composition of fruit powder (FP) and seed powder (SP) were analyzed for the difference between the samples. Maximum moisture was tended in FP (90.11±0.005%) followed by SP (6.28±0.005%). The crude fat was determined as (1.65±0.23%) in FP and (4.82±0.001%) in SP and crude fiber arbitrated means± SD values (2.53±0.13%) in FP and (12.02 ± 0.06%) in SP. Crude protein was found in lesser quantity in FP (1.58±0.013) in comparison of SP which is (12.02± 0.06%). Minerals like magnesium, calcium, potassium, iron and sodium were determined in FP and SP as shown in Table 2.

Table 2: Compositional analysis of fruit (FP) and seed powder (SP) of *Opuntia Ficus Indica*

Compositional Analysis	FP (Means± SD)	SP (Means± SD)
Moisture (%)	90.11±0.05%	6.28±0.005%
Crude fat (%)	1.39±0.23%	4.82±0.001%
Crude fiber (%)	2.53 ±0.13%	4.2±0.06%
Crude protein (%)	1.58± 0.013%	12.02±0.06%
Ash (%)	1.11±0.012%	1.52 ±0.013%
NFE (%)	3.05±0.05%	70.62±0.006%
Mineral analysis of fruit and seed powder of *Opuntia Ficus Indica* (mg/100g)		
Magnesium (Mg)	19.01 ± 0.338	10.04 ± 0.168
Calcium (ca)	12.22±0.204	20.39±0.6
Iron (Fe)	1.503±0.006	0.11±0.006
Sodium (Na)	1.075± 0.023	0.598 ±0.009
Potassium (K)	194.197±1.9	79.87±1.41

Results are presented as mean ± SD; $P<0.05$ significance value followed by Tukey's HSD multiple comparison tests

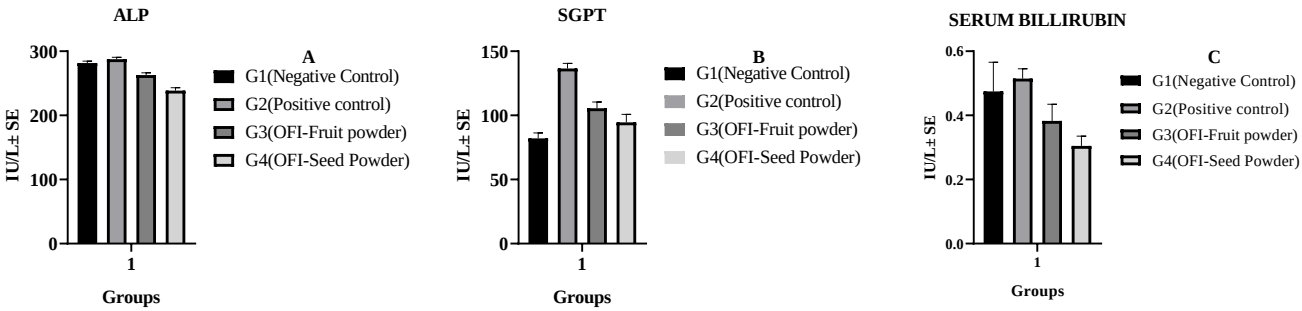

Fig. 1: A) The level of ALP liver enzymes level in all the groups. B) SGPT level in all the treatment group and C) Serum bilirubin level in all the treatment group in comparison to both negative and positive control groups.

Phytochemical screening of *Opuntia Ficus Indica* fruit powder (FP) and Seed Powder (SP) of Via HPLC: Phytochemical and flavonoid screening of *Opuntia Ficus Indica* (OFI) fruit powder (FP) and seed powder (SP) were detected via HPLC. Phytochemicals including quercetin was determined both in seed powder (SP) and fruit powder (FP). It tended for the level of quercetin was 44.077 ± 0.76 mg/100g in SP and 21.855 ± 0.646 mg/100g in FP. However, phenolic acid compounds were detected in fruit powder (FP) and seed powder (SP) such as gallic acid (38.86 ± 1.183 mg/100g), vanillic acid (39.734±1.21 mg/100g), ferulic acid (11.678±0.476 mg/100g) and chlorogenic acid (51.306 ±1.562 mg/100g) showed high quantity in seed powder (SP) and fruit powder(FP) detected lower quantities i.e., gallic acid (33.757 ±1.028 mg/100g), vanillic acid (5.98 ±1.095 mg/100g b), ferulic acid (6.126 b ± 0.240 mg/100g) and chlorogenic acid (12.148±0.369 mg/100g b). p-coumaric acid (5.258 ±0.106 mg/100g), u-coumaric acid (4.733 ±0.144 mg/100g), caffeic acid (12.489 ±0.369 mg/100g). Sinapic acid (53.294±1.575 mg/100g) was high in OFI fruit powder as compared to prickly pear seed powder p-coumaric acid as shown in Table 3.

***Vitamin analysis of Opuntia Ficus Indica fruit powder (FP) and seed powder (SP) via HPLC*:** Vitamins including vitamin C, vitamin E and beta carotene having anti-oxidant potential were determined through HPLC, vitamin C in *Opuntia Ficus Indica* fruit powder (FP) was 39.547± 2.053 mg/100g and 40.69± 0.124 mg/100g in *Opuntia Ficus Indica* seed powder (SP). On the other hand, vitamin E was high in *Opuntia Ficus Indica* seed powder (SP) 296.067± 0.308 mg/100g and fruit powder (FP) showed (9.132± 1.152 mg/100g). Beta-carotene was detected high as *Opuntia Ficus Indica* fruit powder (FP) 355.188 ±1.003 mg/100g and lesser in seed powder (SP) (33.579 ±1.56 mg/100g) as shown in Table 3.

***Serum glutamic pyruvic transaminase/alanine aminotransaminase IU/L, alkaline phosphatase (ALP) IU/L) and serum bilirubin IU/L*:** The G_1 group showed no change in hepatic enzymes while G_2 group showed a significant ($P < 0.05$) (Fig. 1) increase in all the hepatic enzymes after the induction of CCL_4. After 28 days of the study trial, G3 and G4 showed recovery in liver toxicity by lowering the hepatic enzymes close to the normal level of hepatic enzymes as compared to the G2 group.

***Antioxidant and lipid peroxidation on serum markers*:** In the first stage of the study trial, GPx activity was reduced to 0.82% in group G_1 while in G_2 group it was 13.75% reduced (Fig. 2). The GPx activity in group G_3 was increased as 20.84%. However, an increase of GPx level in G_4 group was found as 57.78%. Catalase activity was 3.05% reduced in G1 group and 9.57% reduction was observed in G_2 group. The G_3 group showed 19.69% and G_4 group showed 16.48% activity of catalase. The current study overall showed high level of superoxidase activity 53.91% in G_2 group while G_4 group over all showed low activity 18.13%.

***Total antioxidant capacity (TAC) and total oxidant status (TOS)*:** In Fig. 3 TAC level was increased 2.34% in G_1, and total oxidant status (TOS) was increased as 1.27% in G_2 group. The current results observed that in G_1 group antioxidant potential was improved after intervention trial. Total antioxidant (TAC) capacity was decreased as 10.6% and total oxidant status (TOS) was increased as 13.76% in G_2 group. However, total antioxidant status was significantly improved in G_3 7.73% and total oxidant status (TOS) was decreased as 25.24%. In G_4, TOS was reduced as 25.37% and TAC was increased.

***Inflammatory cytokines (TNF-α, IL-6 and IL-b1)*:** The inflammatory cytokines were analyzed and presented in Fig. 4. Results revealed that G_1 showed less expression level of IL-β1 and IL-6 as 9.67% and 9.41% but a bit rise at the later stages of the study. Tumor necrosis factor alpha (TNF alpha) showed 18.88% rise at the end of trial. On the other hand, G_3 decline in the level of IL-1 β as 13.54%, IL-6 at 8.61%, and sharp decline in TNF-α to 11.39%. While as in G_4 the level of IL-1β is also decline to 11.96%. IL-6 decline to 9.31% and TNF alpha also decline to 26.13%.

***Histopathological examination of liver cells*:** Liver tissue histopathology was analyzed after staining with haematoxylin and eosin under light microscope at 10X amplification. Pathological alterations were observed in the hepatic tissue of G2 group with membrane thickening (multilayering) and infiltration of immune cells. G3 group showed recovered liver parenchyma with no infiltration of immune cells. The histopathological results of G4 group showed low infiltration of immune cells, reduced membrane thickening and recovered liver tissue as compared to G2 group as shown in the (Fig. 5).

Table 3: Phytochemical, vitamin C, vitamin E, and β-carotene screening of *Opuntia Ficus Indica* (prickly pear) fruit (FP) and Seed (SP) Powder through HPLC

Treatments	*Opuntia Ficus Indica* fruit powder (FP) mg/100g	*Opuntia Ficus Indica* seed powder (SP) mg/100g
Quercetin	21.855 ± 0.646	44.077±0.76
Gallic Acid	33.757 ± 1.028	38.86 ±1.183
Caffeic acid	12.489 ± 0.369	10.706 ±0.316
Vanillic acid	35.98 ± 1.095	39.734 ±1.21
Chlorogenic acid	12.148 ± 0.369	51.306 ±1.562
Syringic acid	--	15.97 ± 0.486
P-coumeric acid	5.258 ± 0.106	5.016 ±0.148
U-coumeric acid	4.733 ± 0.144	3.377 ±0.102
Ferulic acid	6.126 ± 0.24	11.678 ±0.476
Cinamic acid	9.797 ± 0.298	--
Sinapic acid	53.294 ± 1.575	5.218 ±0.158
VIT C mg/100g	39.55± 2.053	40.69± 0.124
VIT E mg/100g	9.13± 1.152	296.07± 0.308
β-carotene mg/100g	355.19 ±1.003	33.58 ±1.555

Results are presented as Mean ± SD; P<0.05 significance value followed by Tukey's HSD multiple comparison tests

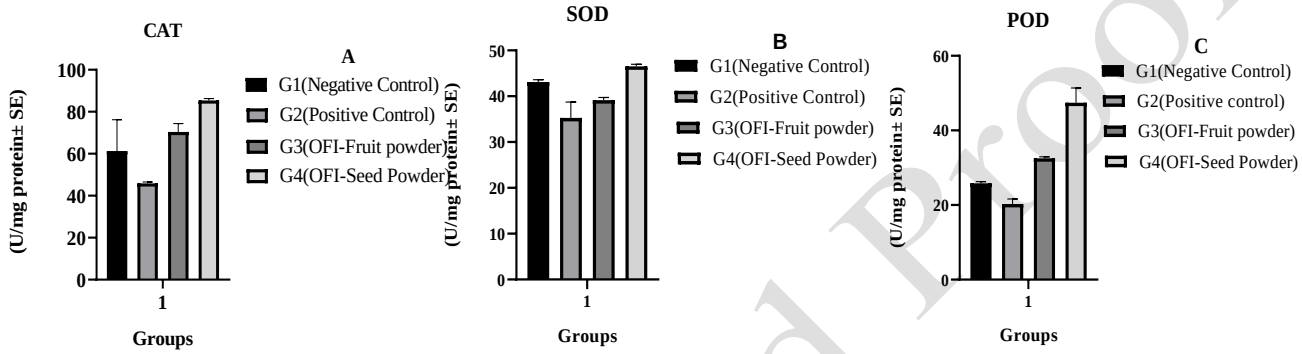

Fig. 2: A) CAT level in all the treatment groups, B) SOD anti-oxidant level in all the treatment group, C) Serum POD level in all the treatments groups in comparison to both negative and positive control group.

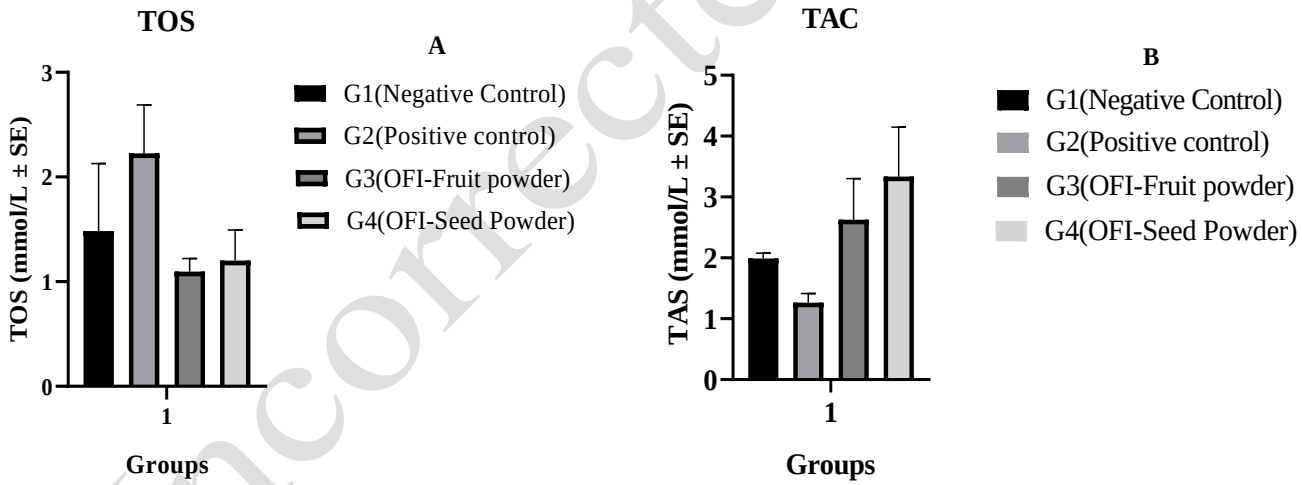

Fig. 3: A) Total oxidative stress level in all the group, B) Total anti-oxidant level in the entire treatment groups in comparison to negative and positive control groups.

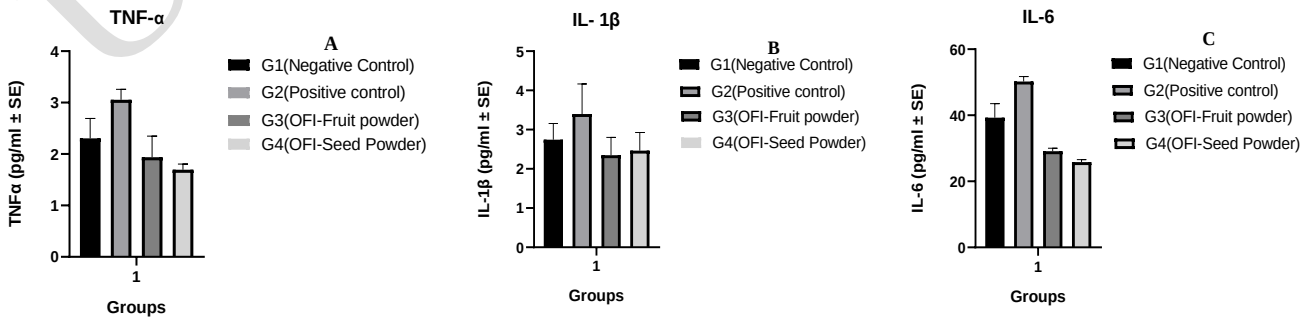

Fig. 4: A) TNF-alpha level, B) Interleukin-1 beta, C) Interlukin-6 level in the entire treatment group in comparison to negative and positive control groups.

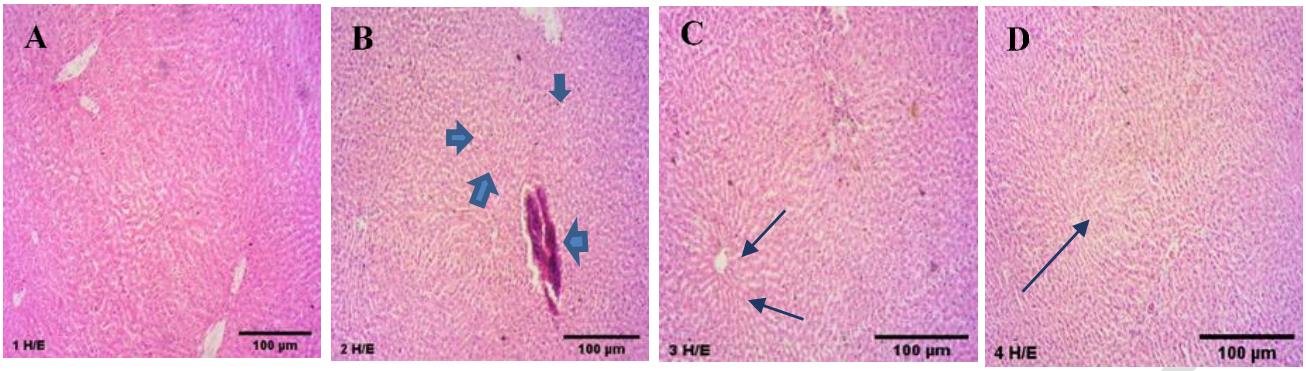

Fig. 5: Photomicrograph of the hepatic parenchymal cells, (A) Negative control no change G_0 (B) Positive control G_1 with membrane thickening (multilayering) and infiltration of immune cells, (C) Treatment group OFI-FP G_2, recovered liver parenchyma with no infiltration of immune cells. and D) Treatment group OFI-SD G_3 showed low infiltration of immune cells (images were taken at magnification power 100X and stained with H and E stain).

DISCUSSION

The present study results showed a significant amount of *quercetin* in OFI seed powder having the potential of scavenging power of free radicals as compared to the OFI fruit powder. Scavenging activity improves the catalase enzyme activity through cessation of the oxidative stress mechanism. Through dicaffeoyl, quinic acid and quercetin-3-O-galactoside have strong free radical scavenging capacities (Beekmann *et al*., 2012; Trendafilova *et al*., 2011).

OFI fruit and seed powder were prepared to analyze the therapeutic potential against hepatotoxicity induced by CCL_4. The acute liver toxicity induced by CCL_4 can cause a significant increase in the serum bilirubin after induction with CCL_4 due to activation of cytochrome P-450 causing damage; inflamed hepatic parenchymal cells as well altered liver enzymes (ALT, AST, and bilirubin level). Seed-powered of OFI possess higher antioxidant levels as compared to OFI pulp-powered (Cheng *et al*., 2013). While as OFI pulp powered has more potential for lowering the synthesis and production of oxidative material and recovering the liver parenchyma. It also plays a critical role in settling the bilirubin and liver enzymes.

Inflammation is a protective response of the body against any physical or chemical insult to the tissue of tissue. Phenolic compounds from the plant origin reduce oxidative stress via increasing the activity of glutathione. It stops the oxidative reaction, the activity of hydrogen peroxide that produces oxidant species, and ceases hydrogen peroxide activity. OFI contains the high number of pigments such as total beta-lain (beta-xanthin and betacyanin) (Cejudo-Bastante *et al*., 2014). Beta-lain (betacyanin and bet-xanthin) ceased the production of hydrogen peroxide species and decreased GPx levels. Both flavonoids (Alves *et al*., 2017) and phenolic acids (Yahia and Mondragon-Jacobo, 2011) possess radical scavenging activity and increased glutathione peroxidase activity as well as a significant role of GPx to reduce the levels of hydrogen peroxides and lipid peroxidation (Ibrahim *et al*., 2018). OFI showed 85.2% cessation of TNF-α as concentration corresponds significant (<0.001). The activity of TNF-α and IL-6 was suppressed with the usage of *opuntia* extract of ethyl acetate as fruit has the quantity of flavonoids and phenolics. In another study, *Opuntia* leaves play a crucial role in immunity especially in inflammatory cytokines such as interleukins IL-6 and TNF-*α*. Their activity stimulates the IL-6 synthesis in several cell types and in turn IL-6 inhibits TNF-*α* production providing negative feedback. Thus, acute inflammatory response was inhibited (Siddiqui *et al*., 2016).

Opuntia leaves has been reported to have the potential to protect the inflammatory cytokines IL-6, IL-1β and TNF-α after induction of phenolics and antioxidant components to stop reactive oxidant species (ROS) after inhibiting the activity of interleukins (Panico *et al*., 2007). Both *opuntia* pulp and seed powder have vitamins, phenolics as well as bio-active components to decrease the activity of inflammatory markers by improving the activity of liver enzymes as regeneration of liver cells happens. Indigenous *opuntia* in current research has quercetin inhibiting the TNF-*α* and IL-6 activity (Antunes-Ricardo *et al*., 2015).

Histopathological examination of liver parenchyma showed a degenerative process of portal areas of the liver with mild infiltration of immune cells in the positive control group in comparison to negative control and both treatment groups OFI-FP, and OFI-SP. Also, the fatty and hydropic changes in the hepatocytes were markedly reduced in both treatment groups of OFI-FP, and OFI-SP. The polyphenols, ascorbic acid, beta-lain, and flavonoids in OFI pulp showed regeneration of the hepatic cells (Chahdoura *et al*., 2014). Oxidative stress dependent inflammation of the hepatic parenchyma and increases the number of mononuclear inflammatory leukocyte (Elgawish *et al*., 2015).

The findings of the current research correspond to the regenerative power of the OFI fruit and promote liver tissue regeneration after damage by CCl4. Induction of CCl4 chemical undergoes a biotransformation of hepatic microsomal cytochrome P-450, to produce trichloromethyl free radical (Singh and Singh 2021). These hepatotoxic metabolites can react with proteins and lipids in the membrane of cell organelles leading to necrosis of hepatocytes (Alimi *et al*., 2012).

Conclusions: Overall study results concluded that the level of liver enzymes was significantly reduced in both of the treatment groups OFI-FP, and OFI-SP due to anti-oxidant dependent anti-inflammatory activity (IL-β1, IL-

6). However, TNF-α efficacy of the OFI pulp and seed extract after induction of phenolics and antioxidant components was found to stop reactive oxidant species (ROS).

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Authors' contributions statement: HH execution data curation and writing-original draft. **BI** conceived the resources, Supervised the work**,** Writing-review & editing. **MSB** Formal analysis **and MNF** conceptualization.

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