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# ANTIFIBROTIC EFFECTS OF ANGIOTENSIN RECEPTOR BLOCKERS IN ORGANOPHOSPHATE (CHLORPYRIFOS) INDUCED LIVER FIBROSIS

**Aftab Abbasi[1], Samreen Memon[2*], Salman Ahmed Farsi Kazi[3]**

[1]Associate Professor, Department of Anatomy, Isra University, Hyderabad - Pakistan
[2*]Professor, Chairperson, Department of Anatomy, Liaquat University of Medical and Health Sciences, Jamshoro - Pakistan
[3]Professor, Department of Forensic Medicine, Isra University, Hyderabad - Pakistan

***Corresponding Author:** Samreen Memon
*Professor, Chairperson, Department of Anatomy, Liaquat University of Medical and Health Sciences, Jamshoro - Pakistan Email: samreen.memon@lumhs.edu.pk

**Abstract**

**Background:** Organophosphates (OPs) are one of the pesticides causing toxicity to humans and animals. OPs toxicity involve many organs such as liver, kidney, nervous system and immune system. Liver fibrosis is a wound healing response in which there is increased production and deposition of extracellular matrix (ECM), due to prolonged injury to liver by viral hepatitis, alcohol consumption, metabolic diseases and toxins (pesticides). Angiotensin receptor blockers (ARBs) are potential therapeutic agents for liver fibrosis.

**Objectives:** To determine the effects of ARBs on Chlorpyrifos(CPF) induced liver fibrosis.

**Methods:** 40 adult male albino wistar rats were used in the study. Rats were grouped as control group (A1), positive control group (A2) and experimental groups (B, C and D). Group A2 was treated with carbon tetrachloride (CCL4) to induce liver fibrosis, with 2 ml/kg of 50% solution of CCL4 by oral gavage two times per week for eight weeks, group B was treated with 1ml/bw/day CPF intraperitoneally for eight weeks. Group C was treated with 1ml/bw/day CPF intraperitoneally for eight weeks and then ARBs orally (30mg/kg/day) for 8 weeks. Group D was simultaneously treated with CPF and ARBs for 8 weeks with same doses as for group C. At the end of experiment blood was collected by cardiac puncture for liver function test and rats were sacrificed to collect liver samples. Pathological changes in liver were examined using H&E and Masson's trichrome staining. Real time PCR was used to detect alpha smooth muscle actin (α-SMA), transforming growth factor beta 1(TGF- β1) and Collagen-I(coll-I) mRNA expression.

**Results:** CPF caused liver injury evidenced by raised liver enzymes and bilirubin with histological changes and increased expression of genes. ARBs decreases liver enzymes, CPF induced histological changes and decreases the expression of α-SMA, TGF- β1 and Coll-I.

**Conclusion:** The present study suggests that ARBs prevent fibrogenesis and may serve as a promising therapeutic agent to reduce CPF induced liver fibrosis.

**Key Words:** Liver fibrosis, chlorpyrifos, angiotensin receptor blockers, collagen, alpha smooth muscle actin.

## 1. INTRODUCTION

Liver fibrosis is a critical pathogenic and healing process, developed by repetitive injury to normal liver tissue, due to various infectious agents, toxicity of drugs, metabolic and immune diseases (1). It is a type of wound healing response that results from chronic injury to liver irrespective of the underlying cause (2). Hepatic stellate cells (HSCs) are nonparenchymal cells in the sinusoidal space, these cells are activated to form myofibroblast like cells. This activation is the key step in development of liver fibrosis (3). The activation is stimulated by toxins, viral hepatitis, autoimmune diseases and steatohepatitis (4). Profibrotic cytokines, like TGF-β is one of the common cell mediator formed by various liver cells after any kind of damage (5).

TGF-β controls growth and differentiation of cells, movement of cells, formation of ECM, formation of new blood vessels and mediate immune responses of cells (6). TGF-β promotes the transformation of HSCs into myofibroblast-like cells, boosts the synthesis of ECM proteins and inhibits the degradation of ECM proteins in vivo. In experimental models, strategies aiming at inhibiting TGF-β production or signaling pathways resulted in a significant reduction in fibrosis (7). α-SMA acts as a marker of the cells causing organ fibrosis. Activated myofibroblasts with α-SMA expression play a notable role in the process of organ fibrosis. Detection of α-SMA gene expression is an effective step in monitoring the differentiation of myofibroblast in order to treat fibrosis (8). Collagen I is a hallmark of stimulated HSCs and it is one of the major component of ECM. α-SMA along with a role in contraction of HSCs, also controls the expression of collagen I by conveying mechanical signals to nucleus (9).

Pesticides are chemicals deliberately introduced into environment and they become an essential part of farming nowadays to increase the agricultural output by limiting losses incurred by pests. Excessive and inappropriate application of pesticides is one of the serious health and environmental issue worldwide (10). OPs compounds are one of the type of pesticides that intend to kill insects and therefore are known as insecticides. Exposure to these insecticides to community occur while application in agricultural fields, by intake of food or when pesticides are sprayed in or around the house (11). Literature showed that OPs toxicity is attributed not only to cholinesterase inhibition but also by excessive production of free radicals, causing oxidative stress (12). This oxidative stress may produce abnormal functioning of organs. In liver they produce impairment in architecture, metabolic and chemical processes diagnosed by altered levels of serum bilirubin and aminotransferases (13, 14). Studies have confirmed that the liver tissue is the major organ affected by OP poisoning. Chlorpyrifos is a typical representative of the OPs, which have negative effects on both liver function and liver structure. Mikhail et al (15) discovered nearly four decades ago that a two-day intraperitoneal exposure to a sub-lethal dose of this pesticide resulted in mid-zonal liver necrosis, fatty deposition in the periphery and glycogen deposition on one side of the hepatic cell and around the central vein.

The renin-angiotensin system (RAS) is a physiological regulator of blood pressure, electrolyte balance and fluid homeostasis (16). The RAS precursor angiotensinogen (AGT) and its cleavage enzyme renin, have been extensively detected in normal and in injured liver tissue. Levels of angiotensin converting enzyme (ACE) and angiotensin type 1 receptors (AT1R) are remarkably increased after liver injury, particularly in fibrotic areas of the liver (17). Researchers have discovered that when angiotensin II (Ang II) is delivered to the liver through AT1R, HSCs grow more rapidly and develop contractile features (18). Inhibition of Ang II activity by Lisinopril (an ACE inhibitor), Losartan (an AT1R antagonist), N-acetylcysteine and diphenyleneiodonium (Nox inhibitors) inhibited RAS induced pro-fibrogenic effects (19). The effectiveness of RAS inhibitors in randomized controlled trials in patients with liver fibrosis was recently reviewed and a significant reduction in fibrosis score was observed (20). Presently no data is available on preventive effects of ARBs on CPF induced liver fibrosis. This study is designed to see the effects of ARBs on CPF induced liver fibrosis among rats.

## 2. MATERIAL AND METHODS

**2.1 Preparation of chemicals:** Carbon tetra Chloride (CCL4) was purchased from BDH chemicals(UK), 50% v/v solution of CCL4 was prepared by mixing 2.5 ml of CCL4 and 2.5 ml of olive oil for 5 rats. Solution was freshly prepared at the time of administration (21). OP (CPF) was purchased from Jaffer group of companies (Pakistan). The stock solution of 25 ml was prepared by mixing 2.5ml of CPF and 22.5ml distilled water (22).

### 2.2 Experimental Animals and Treatments

40 adult male albino wistar rats of 250-300gm purchased from Sindh Agriculture University Tandojam, were housed under standard conditions and supplied chow diet and water ad libitum. 12 hour light/12 hour dark cycles were maintained in the room where rats were housed. After one week of acclimatization the rats were weighed and divided into 5 groups. Control groups contain 5 animals, all other groups contain 10 animals (23).

During the experiment, body weights were regularly monitored and the doses of experimental therapeutics were adjusted accordingly. Survival and clinical signs of intoxication were also evaluated on a daily basis.

### 2.3 Determination of Liver Function Markers

The blood samples were collected at the end of experiment by cardiac puncture for liver function test (24). Serum glutamate pyruvate transaminase(SGPT), gamma glutamyl transferase(GGT), alkaline phosphatase(ALP) and total bilirubin were assessed using commercially available assay kits (CobasC311 analyzer).

**Table No: 1 Experimental design**

| Group | No. of Animals | | Treatments |
|---|---|---|---|
| **A1** | 5 | Control group | Water and chow diet |
| **A2** | 5 | Positive control group | 2 ml/kg of 50% solution of CCL4 orally two times/wk for 8 weeks.(21) |
| **B** | 10 | Treatment group | 1ml/bw/day CPF intraperitoneally(I/P) for 8 weeks (22) |
| **C** | 10 | Treatment group | CPF (same dose and duration as for group B) and then ARB (losartan) orally (30mg/kg/day) for 8 weeks (25) |
| **D** | 10 | Treatment group | CPF+ARB (same doses as for group C) |

### 2.4 RNA isolation and Quantitative Real Time Polymerase chain reaction (qRT-PCR)

Total RNA was extracted from frozen samples of liver(30mg) by following the protocol of favorgon RNA extraction kit. The isolated RNA was quantified and Purity of extracted sample was checked using nanodrop 1000 spectrophotometer (Thermoscientific, USA). qRT-PCR and cDNA synthesis was carried out using "Superscript III platinum SYBR green one step qRT PCR kit with ROX". Reactions were set up on ice. Primers used in the study were listed in table 2 (21, 26). PCR plate was prepared and mixed gently by tapping tubes. PCR plate was sealed and placed in preheated real time instrument programmed as follows, cDNA Synthesis at 50°C for 3 minutes hold, PCR at 95°C for 5 minutes hold. 40 cycles consist of three steps of PCR. Denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds and Extension at 40°C for 1 minute. Relative quantification of mRNA expression was performed by "Applied Biosystems 7300" for real time PCR.

**2.5 Histopathology:** The liver tissues were preserved in 10 % formalin then processed for paraffin embedding. After sectioning 4-μm slices were stained with hematoxylin and eosin (H&E) and Masson's trichome to detect liver fibrosis using light microscope (27, 28).

**2.6 Statistical Analysis.** Data was statistically analyzed using SPSS version 22.0. The quantitative data were presented as means ± standard deviation (SD). The statistical comparisons were made by

one-way ANOVA followed by Scheff'e test. The qualitative data was analyzed by chi square test. *p*-value < 0.05 indicates a statistical significance.

**Table:2** Primers used for qRT-PCR

| **Gene** | **Forward primer Sequence**(5'-3') | **Reverse primer Sequence**(5'-3') |
|---|---|---|
| **α-SMA** | GAACACGGCATCATCACCAA | CAAGGTCGGATGCTCCTCTG |
| **Coll I** | CTCCTGGCAAGAACGGAGA | CCAGCTGTTCCAGGCAATC |
| **TGF-β1** | CCGCAACAACGCAATCTATG | AGTTCTACGTGTTGCTCCACAGT |
| **Gapdh** | AGTGCCAGCCTCGTCTCATA | GATGGTGATGGGTTTCCCGT |

Gapdh, glyceraldehyde-3-phosphate dehydrogenase (Internal primer), α-SMA, alpha smooth muscle actin, Col I, collagen I, TGF-β1, transforming growth factor beta 1

## 3. RESULTS

### 3.1 Effect of ARBs on body weight of animals

The mean ± SD of body weight in the groups A1, A2, B, C and D was noted. The animals had initial weights between 250 to 270 grams, the average weights among all groups had no significant difference. At the end of experiment, the control group had highest average weight followed by group D and the lowest average weight was recorded for group A2, 212.20±5.26 grams and group B, 210.60±6.36 grams (Table 3). For post-hoc analysis the Scheff'e test was used to find particular group differences. Each group's mean and SD were computed and pairwise comparisons were performed. The Scheff'e test significance threshold was set at *p*-value <0.05. The Group B (CPF induced) was found to be statistically different from groups A1, C and D, indicating that CPF caused significant weight reduction in the experimental animals. The ARBs treated groups C and D showed less pronounced weight reduction with a statistically significant difference in body weight compared to A1, A2 and B.

**Table 3.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on weight of experimental rats in grams and pairwise comparison by Scheffé test

| **Group** | **Animal weight (gm)** | | **(*p*- value <0.05)** |
|---|---|---|---|
| | **Mean** | **±SD** | |
| A1 | 266.60 | 7.40 | A2, B, C, D |
| A2 | 212.20 | 5.26 | A1, C, D |
| B | 210.60 | 6.36 | A1, C, D |
| C | 245.80 | 10.05 | A1, A2, B |
| D | 250.30 | 8.56 | A1, A2, B |

**p*- value ≤0.05 was considered significant

### 3.2 Effect of ARBs on liver weight

The average mean liver weight was found highest for control group and group D, showing protective role of ARBs against CPF in group D with no statistically significant difference between these groups, while the lowest average weight was found in group A2, B and C respectively. (Table 4)

**Table 4.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on liver weight of experimental rats in grams and pairwise comparison by Scheffé test

| | **Liver weight (g)** | | **(*p*-value <0.05)** |
|---|---|---|---|
| **Group** | **Mean** | **±SD** | |
| A1 | 5.06 | 0.207 | A2,B,C |
| A2 | 3.32 | 0.83 | A1,D |
| B | 3.46 | 0.41 | A1,D |
| C | 3.82 | 0.2530 | A1 |
| D | 4.30 | 0.3399 | A2,B |

**p*- value ≤0.05 was considered significant

### 3.2 Effect of ARBs on liver function test

On liver function test the total bilirubin was measured. The ANOVA conducted on the data of total bilirubin revealed statistically significant differences among the groups. The lowest bilirubin was recorded for group D, almost equal to control group showing no hepatic injury in this group and the highest one was recorded for groups A2 and B. The experimental group C (ARBs after fibrosis induction) shows lower averages of bilirubin as compared to group B with more profound reduction in serum bilirubin in group treated with combination of ARBs and CPF, group D (Table 5). The liver enzymes SGPT, GGT and ALP were also reduced in group D, almost equal to control group showing simultaneous administration of ARBs with CPF prevent fibrosis generation as compared to the group C in which ARBs administered after fibrogenesis. (Table 6)

**Table 5.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on mean values of total bilirubin in experimental groups and pairwise comparison by Scheffé test

| **Group** | **Total Bilirubin(mg/dl)** | | **(*p*-value <0.05)** |
|---|---|---|---|
| | Mean | ±SD | |
| A1 | 0.15 | 0.042 | A2, B, C |
| A2 | 0.5 | 0.038 | A1, C, D |
| B | 0.46 | 0.033 | A1, C, D |
| C | 0.38 | 0.027 | A2, B, D |
| D | 0.25 | 0.030 | A2, B, C |

*$p$- value ≤0.05 was considered significant

**Table 6.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on SGPT, GGT and ALP levels in experimental groups

| **Group** | **SGPT(IU/L)** | | **GGT(IU/L)** | | **ALP(IU/L)** | |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Mean | ±SD | Mean | ±SD |
| A1 | 38.5 | 2.61 | 3.8 | 0.795 | 81.4 | 2.302 |
| A2 | 173.5 | 9.56 | 17.8 | 2.588 | 174.6 | 10.644 |
| B | 168.6 | 7.88 | 16.1 | 1.728 | 168.8 | 10.454 |
| C | 78.22 | 5.64 | 14.5 | 2.173 | 150.9 | 8.4123 |
| D | 62.52* | 4.58 | 4.7* | 1.264 | 120.2* | 5.2662 |

*Serum mean values of SGPT, GGT and ALP in group D are almost equal to control group(A1)

### 3.3 Effect of ARBs on liver fibrosis

The protective effects of ARBs also supported by histological examination of liver using H&E and Masson's trichome staining as shown in figures 1-8. The histopathological staging of fibrosis was done on the basis of Ishak scoring system. The Ishak score is a widely used histological scoring system that evaluate the severity and stage of liver fibrosis. It provides a numerical value to access the degree of fibrosis based on histopathological features observed in liver tissue sample. The Ishak score ranges from 0-6, with 0 indicating no fibrosis, 1- fibrous expansion of some portal area with or without fibrous septa, 2- fibrous expansion of most portal area with or without fibrous septa, 3- fibrous expansion of most portal area with occasional portal to portal bridging, 4- fibrous expansion of portal area with marked bridging portal to portal as well as portal to central, 5- marked bridging with occasional nodules, 6- cirrhosis(29). The H&E and Masson's trichome staining showed normal histology of liver in control group while obvious fibrosis was observed in CPF induced group B. Ishak score showed the presence of stage 1, 2 and 3 liver fibrosis in group B. Combination treatment with CPF and ARBs in group D significantly decreases fibrotic score, proving protective effects of ARBs in fibrogenesis. (Table 7) (Figure 1 -8).

**Table 7.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on histological staging of liver fibrosis in experimental groups

| Group | Histological staging | | | | | |
|---|---|---|---|---|---|---|
| | **Normal** | **Stage 1** | **Stage 2** | **Stage 3** | **Stage 5** | **Total No. of liver samples** |
| A1 | 5 | 0 | 0 | 0 | 0 | 5 |
| A2 | 0 | 0 | 0 | 3 | 2 | 5 |
| B | 0 | 3 | 2 | 5 | 0 | 10 |
| C | 0 | 4 | 6 | 0 | 0 | 10 |
| D | 5 | 5 | 0 | 0 | 0 | 10 |

Histological staging of liver fibrosis showed no significant difference between group D and control group showing inhibitory effect of ARBS on CPF induced fibrogenesis. (0-no fibrosis, 1- fibrous expansion of some portal area with or without fibrous septa, 2- fibrous expansion of most portal area with or without fibrous septa, 3- fibrous expansion of most portal area with occasional portal to portal bridging, 4- fibrous expansion of portal area with marked bridging portal to portal as well as portal to central, 5- marked bridging with occasional nodules, 6- cirrhosis)

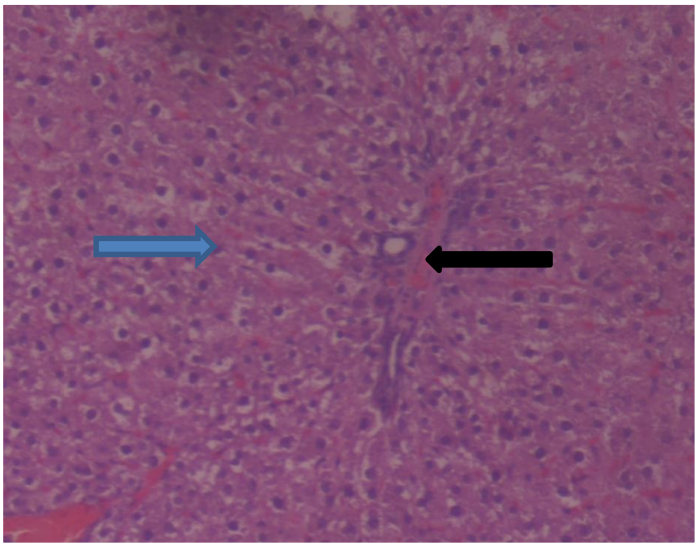

**Figure 1.** Photomicrograph of 4um Thick H&E Stained Sections of Liver Showing Normal Architecture with Normal Portal Triad (black arrow) and Hepatic Plates (blue arrow) X 100 magnification (Control group)

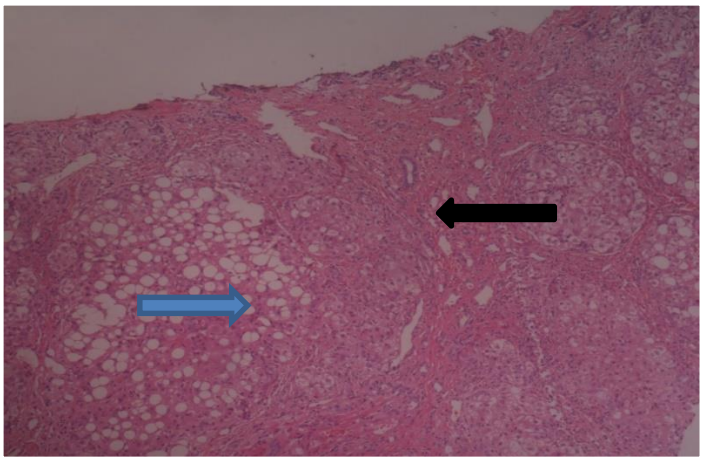

**Figure 2.** Photomicrograph of 4um Thick H&E Stained Section of Liver Showing Stage 5 Fibrosis with Portal-Portal Bridging (black arrow) Nodule Formation and Vacuoles (blue arrow) X 100 magnification (Positive Control Group)

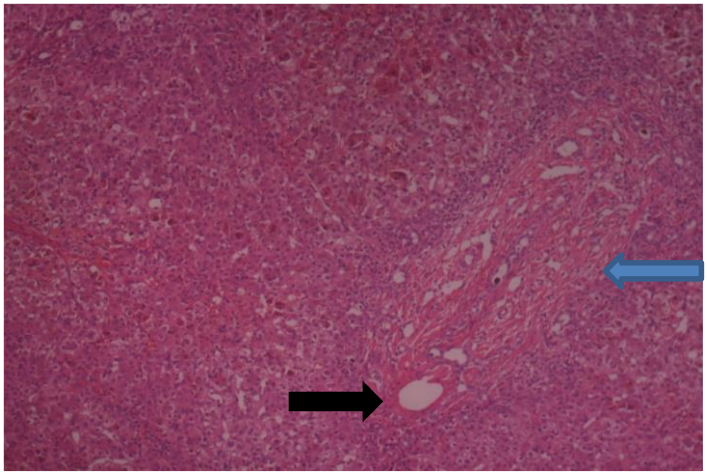

**Figure 3.** Photomicrograph of 4um Thick H&E Stained Section of Liver Showing Stage 3 Fibrosis with Expansion of Portal Area (black arrow) and Portal–Portal Bridging (blue arrow) X 100 magnification (Group B)

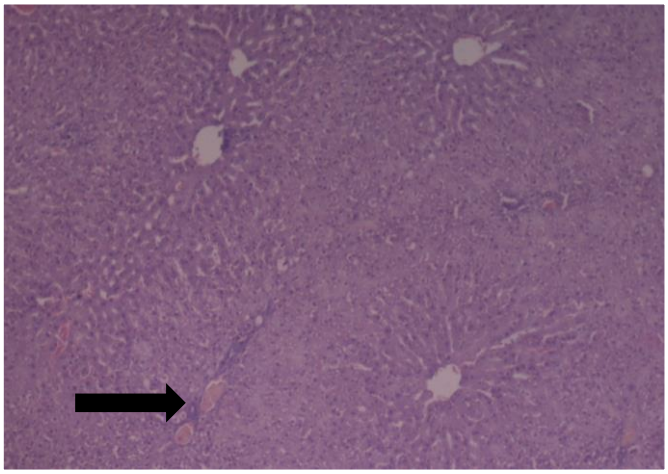

**Figure 4.** Photomicrograph of 4um Thick H&E Stained Section of Liver Showing Stage 1 Fibrosis with fibrous expansion of some portal areas (black arrow) X 100 magnification (C Group)

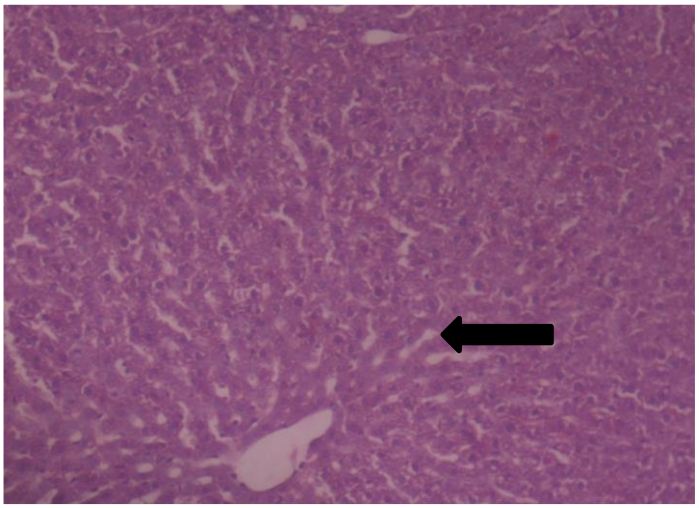

**Figure. 5** Photomicrograph of 4um Thick H&E Stained Section of Liver Showing Almost Normal Histoarchitecture of Liver with Normal Arrangement of Hepatic Plates (black arrow) X 100 magnification (D Group)

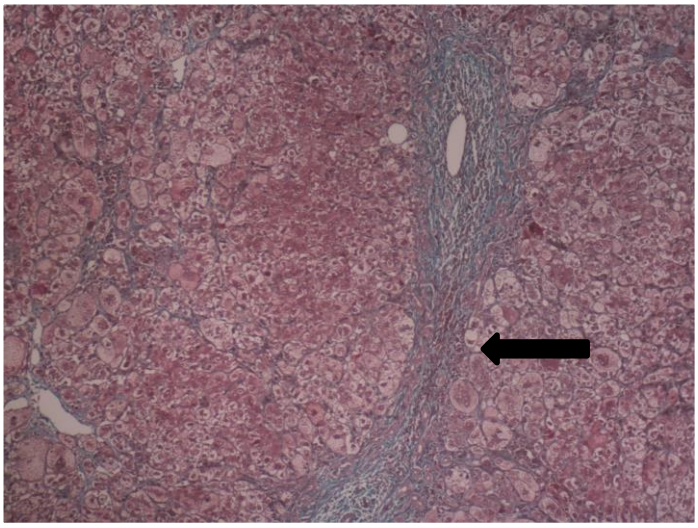

**Figure. 6** Photomicrograph of 4um Thick Masson's Trichome Stained Section of Liver Showing Stage 3 Fibrosis with Portal-Portal Bridging (black arrow) X 100 magnification (Group B)

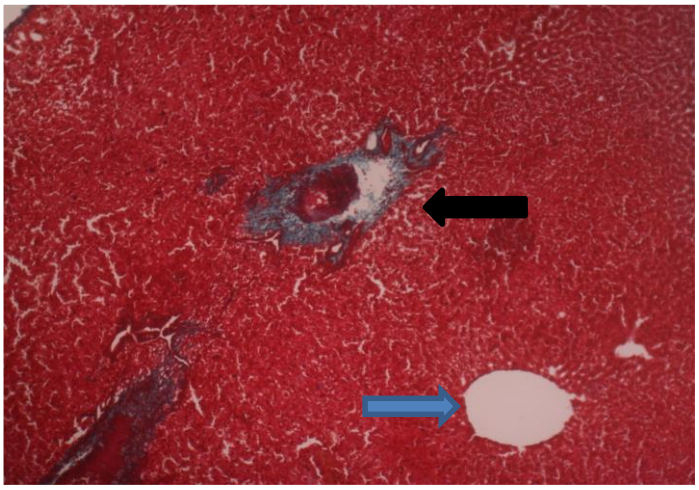

**Figure. 7** Photomicrograph of 4um Thick Masson's trichome Stained Section of Liver Showing Stage 2 Fibrosis with Dilatation of Portal Vein (black arrow) and Dilatation of Central Vein (blue arrow) x 100 magnification (C group)

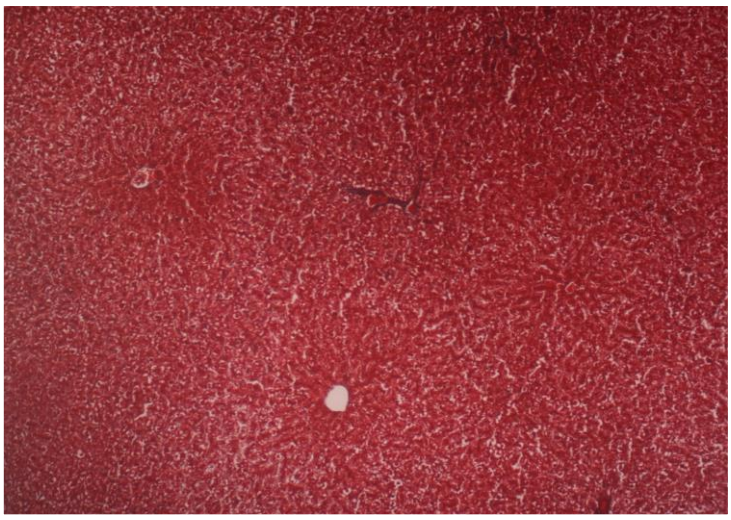

**Figure. 8** Photomicrograph of 4um thick Masson's trichome Stained Section of Liver Showing No fibrosis with Almost Normal Histoarchitecture X 100 magnification (D group)

### 3.4 Gene expression analysis

Gene expression analysis of three genes, α-SMA, TGF-β1 and coll –I showed lowest expression of all genes in group D, followed by group C which also supported the protective effects of ARBs on CPF induced liver fibrosis (Table 8)

**Table 8.** Effects of CPF (group B), ARBs after fibrogenesis by CPF (group C) and ARBs + CPF (group D) on gene expression analysis of α-SMA, Coll-I and TGF- β1 in experimental groups

| | **α-SMA** | | **Coll-I** | | **TGF- β1** | |
|---|---|---|---|---|---|---|
| **Groups** | **Mean** | **SD±** | **Mean** | **SD±** | **Mean** | **SD±** |
| A1 | 1 | 0.08 | 1 | 0.09 | 1 | 0.059 |
| A2 | 3.72 | 0.16 | 4.83 | 0.1902 | 4.79 | 0.14 |
| B | 2.55 | 0.24 | 3.82 | 0.1523 | 3.64 | 0.098 |
| C | 1.33 | 0.39 | 1.67 | 0.1313 | 2.61 | 0.19 |
| D | *1.07 | 0.13 | *****1.31 | 0.1951 | *****1.21 | 0.41 |

* This table shows decreased expression of genes (α-SMA, Coll-I and TGF-β1) in group D.

## 3. DISCUSSION

The pharmaceutical and chemical industries have experienced significant growth in recent decades, leading to increased production and utilization of chemical compounds as pesticides in agriculture and related fields. According to the World Health Organization, approximately three million workers in developing nations face severe pesticide poisoning each year, and around 18,000 of them will ultimately lose their lives due to these toxic exposures(30). OPs have been identified as pesticides that can induce harmful effects when extensively used. Excessive utilization of OPs in both agricultural and industrial sectors may result in severe adverse consequences, posing risks to not only animals but also human health(31). Persistent exposure to these pesticides has been associated with the emergence of liver conditions, such as hepatitis, fibrosis, and cirrhosis(32).

Many western countries have ceased the usage of this compound as pesticide(33). However in developing countries the usage is still above limit causing severe organophosphate poisoning(34). Despite of international data countries like Pakistan are still not stopping the use of pesticides. These being the leading-agents in this region are enhancing the mortality and morbidity rate by misuse and over use(35-37). In the last twenty years, the usage of diverse pesticides in Pakistan has experienced a notable rise. Importantly, approximately 71% of Pakistan's pesticide market depends on imported products, with an annual importation of around 80,000 tons. Presently, Pakistan utilizes approximately 130,000 metric tons of pesticides(38). The data related to poisoning by OP is limited and scarcely available in Pakistan. In this context are either case studies or single setting studies providing limited information on the above subject(39). A hospital study from Karachi in Pakistan showed that about 1900 cases of ICU making a prevalence of 40 percent were due to OP poisoning(40). The overall frequency of mortality was 5.6 percent related to pesticide poisoning only in Pakistan city wise data(41). The present study was designed to evaluate the role of ARBs against the liver fibrosis caused by OP(CPF) poisoning in albino rats. The study was designed for providing a reversal or inhibitory mechanism for treating hepatic poisoning in animals taken as model. Literature supports that OPs can be absorbed into all tissues of animals and can cause various severe pathological problems. The reason for this is the lipophilic nature of OPs leading to their rapid and easy intestinal absorption and assimilations. This causes insufficiency in immune responses, pancreatitis and renal issues as well as hepatic fibrosis as observed in current research(42-46). OP is known agent of xenobiotic-biotransformation. Due to this reason hepatic gene regulators might play the main role in adaptation response generation towards altered metabolism such as by converting enzyme capacities in various related metabolic-pathways(47). As the hepatic system is the principal site for metabolic processes and mediator in the biotransformation of OPs, precipitates fibrogenesis and eventual onset of cirrhosis(48).

In our current study, ARBs showed antifibrotic effect in both group C and D. Moreover, the concomitant use of ARBs and CPF demonstrated greater effect (group D) as compared to use of ARBs after induction of fibrosis (group C). It was shown that CPF poisoning results in significant

decrease in the weight of rodents. It is a sensitive predictor of toxicity and correlated with the histopathological alterations. Hepatotoxicity is reported with increased SGPT, GGT, ALP levels as also observed in present study results. Bilirubin is an excretory end-product of the degradation of heme molecules. It is present in conjugated form inside the liver to produce bilirubin-diglucuronide(49). OPs can cause impaired hepato biliary functions which is an indication of liver damage and fibrosis(13). In scenarios where bilirubin increases with hepatic disease is a result of inflammation and blockage of biliary ducts related to various staging of fibrosis as also reported in current research. The use of ARBs significantly reduces bilirubin levels showing protective role against CPF toxicity(50). The use of ARBs remarkably prevents histopathological and enzymatic changes in group D proved by greatly decreased liver enzymes along with improvement in architecture of liver, showing that simultaneous administration of ARBs and CPF prevent fibrosis generation. The findings of present study are in favor of previous research, where similar results were observed. The investigation reports of study also showed decreased levels of serum SGPT, GGT and ALP along with significant reduction in fibrotic score, upon treatment with losartan in rat model of thioacetamide induced liver fibrosis(51). Another study by **Murad H, et al in 2020** further reinforces the outcomes of present study, where experimental rats exhibited significant rise in ALT and AST enzymes along with evident histopathological damage induced by thioacetamide and administration of ARBs effectively reversed these alterations(52). These consistent results emphasize the capacity of ARBs in improving hepatic injury and reducing inflammatory responses in experimental models. The current study further indicates that ARBs suppresses the synthesis of collagen in response to CPF. The findings are in consistent with previous research in which mice lacking AT1R showed minimal fibrotic lesions(53). These findings suggest that the absence or blockage of AT1R play a protective role against development of fibrosis.

In a study by **Fujinaga Y, et al, 2020** it was observed that renin Ang-II signaling pathway is a crucial mediator in the pathogenesis of fibrosis by regulating the TGF-β1 production, which stimulates HSCs and promotes fibrosis. Liver fibrosis causes activation of HSCs which is associated with the expression of the SMA. These cells are further responsible for enhancement of type-1 collagen production as observed in liver-fibrogenesis(54, 55). TGF-β has also been reported as a significant profibrinogen cytokine which is involved in above process and transcriptionally assist in regulation and expression of type-1 collagen inside the stellate-cells(56)However, the use of ARBs, which act as competitive inhibitors of Ang-II receptors downregulates hepatic α-SMA and TGF-β1 levels.(57) These findings are in consistent with our study, which also showed that ARBs treatment reduced hepatic α-SMA, TGF-β1 and Coll-I gene expression and consequently liver fibrosis. Another study confirmed the antifibrotic potential of ARBs. The study demonstrated that ARBs through their ability to suppress synthesis of collagen and TGF-β production effectively reduce fibrotic process.(58)

Previous reports showed that treatment with ARBs improves hepatic fibrosis by inhibiting HSCs activation. AT-II signaling take an important part in fibrosis by regulating TGF-β1 production which increases fibrosis by stimulating HSCs. ARBs decreases hepatic α-SMA and TGF-β1 levels(57). In our current study, we found that ARBs treatment reduced hepatic α-SMA**,** TGF-β1 and Coll-I and consequently liver fibrosis. An important finding of current study is that ARBs significantly reduce fibrosis when given concomitantly with the agent causing liver injury as CPF in this study as compared to administration of ARBs with established fibrosis.

## 4. CONCLUSION

OPs induced fibrotic disease of liver is of great health care concern in developing countries. This study demonstrated that both concomitant and subsequent use of ARBs with CPF has inhibitory and reversible effect on liver fibrosis respectively. Hence ARBs may be a promising novel approach in prevention and treatment of CPF induced liver fibrosis**.**

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