

# Toxicity studies and anti-arthritic effect of mandelic acid (2-hydroxy - 2-phenyl acetic acid) using *in vitro* and *in vivo* techniques

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**Abstract:** The major concern to search for new anti-arthritic drugs is primarily to prevent systemic complications and to maintain quality of life. As these drugs are prescribed for long duration so the objective is to ensure their safety in terms of toxicity. By keeping in view this concept, the present study was investigated to determine new anti-arthritic potential using *in-vitro* and *in-vivo* methods. The *in-vitro* tests comprised of protein denaturation (BSA and egg albumin) and Human Red Blood cell (HRBC) membrane stabilization assays at 50-6400µg/mL, for *in-vivo* testing, formaldehyde-induced arthritic rats were treated with 40, 80 and 160mg/kg mandelic acid. Mandelic acid (MA) inhibited the protein denaturation and stabilized the membrane of HRBC in a concentration dependent manner. Likewise, mandelic acid exhibited dose dependent reduction in paw volume induced by formaldehyde. For acute and sub-acute treatment, MA did not show any sign of toxicity and mortality in each rat and LD<sub>50</sub> might be greater than 2000mg/kg. In addition, histopathological assessment presented slight increased interstitial spaces in the kidney, disorganization of glomerulus, dilated sinusoids at highest dose 800mg/kg which were not observed in sub-chronic therapy. Hence, these results conclude that mandelic acid has the potential to treat rheumatoid arthritis with observed no significant signs of toxicity and should be tested further to determine anti-arthritic mechanism of drug action at cellular level.

**Keywords:** Anti-arthritic, protein denaturation, mandelic acid, sub-acute toxicity.

## INTRODUCTION

Rheumatoid arthritis (RA) is a debilitating inflammatory autoimmune disorder that involves systemic complications such as bone distortion or damage, augmented morbidity and mortality (Calabresi *et al.*, 2018). Patients with RA have a reduced life quality (joints and bones degeneration, muscle weakness, persistent pain) and to acquire normal life, they have to use anti-rheumatic drugs persistently. A common effect of long-term therapy is the development of resistance against treatment and an increased occurrence of toxic effects in organs. Due to these reasons, a continuous need for new agents in the therapy of RA is envisaged (Gardi *et al.*, 2015).

Quantitative SAR has a potential for designing lead compounds with robust physicochemical possessions as a function of structural groups (Nantasenamat *et al.*, 2009). Literature indicates that phytochemicals due to their distinct properties provide effective potentials against different forms of arthritis including inflammatory, infectious and autoimmune abnormalities (Venkatesha *et al.*, 2016). Among the secondary constituents that comprised of phenolic, terpenes, alcohols and alkaloids, help against biological abnormality and promote defense

system against pathogens, predators and competitors. In ancient times phytochemicals have been used in folk medicines as herbal product and recently in pharmaceutical industries to develop special dosage forms with supported safety, efficacy and quality (Raj *et al.*, 2020).

*Aesculus indica* fruit is widely used in folkloric medicine against oxidative degenerative diseases and inflammation. The main constituents of *Aesculus indica* are mandelic acid and quercetin (Zahoor *et al.*, 2018). Anti-oxidant and anti-inflammatory activities of quercetin and mandelic acid had formerly been reported (Gordon and Roedig-Penman, 1998) and it was determined that these activities might be due to the presence of mono-carbonyl group (Arshad *et al.*, 2017). So, this primary study was piloted to evaluate the anti-arthritic prospective of mandelic acid using *in vitro* techniques and *in vivo* animal model. Furthermore, to determine its potential toxicity in terms of higher doses and continuous therapy, toxicological assays were performed to assess the acute, sub-acute and sub-chronic toxicity in rats.

## MATERIALS AND METHODS

### Chemicals and drugs

Aspirin (Sigma-Aldrich, Steinheim USA), mandelic acid (Sigma-Aldrich, USA), egg albumin (fresh hen's egg),

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diclofenac sodium (Sigma-Aldrich, Steinheim USA), sodium chloride, BSA, Disodium hydrogen phosphate (Sigma-Aldrich), potassium dihydrogen phosphate (Reidel-de-haen, USA), Formaldehyde (VWR, England), Methanol, Ascorbic acid, Naproxen, Sodium hydroxide (Sigma-Aldrich, USA).

### Investigational Animals

Sprague Dawley rats (150-300g) were used for experimental protocols. Animals were retained in animal house of Department of Pharmacology, University of Sargodha, Sargodha with air conditioned temperature. Animals were fed on standard pellet diet and were treated with the procedures of national research council (Council, 2010). The procedure used in present study for animals was permitted by Postgraduate Animal Ethics Committee, College of Pharmacy, University of Sargodha (with approved reference No. SU/Pharm/Animal Ethics Approval/2019/215).

### Anti-arthritic tests

#### Inhibition of denaturation of protein by using egg albumin method

Assay mixture contained 2.8mL of phosphate buffer (pH 6.4), egg albumin (0.2mL) and 2.0mL of different concentrations of 50, 100, 200, 400, 800, 1600, 3200, 6400µg/mL of mandelic acid and aspirin as a reference standard respectively. Thereafter mixture were incubated for 15 minutes at 37°C in an incubator and then placed in a water bath for 5 minutes at 70°C. Afterwards mixture was cooled to room temperature and then absorbance was determined using UV-Visible spectrophotometer at 660 nm (Ahsan *et al.*, 2021). The experiment was repeated three times independently. Percent reduction in protein denaturation was measured by under mentioned equation

$$\left[ \text{Inhibition (\%)} = \frac{\text{Abs. of arthritic Control} - \text{Abs. of test samples}}{\text{Abs. of arthritic Control}} \times 100 \right]$$

#### Inhibition of denaturation of protein using BSA

According to this technique, test substance (0.5mL) comprised of 0.45mL of BSA (0.5%) and 0.05mL of several concentrations of MA as mentioned in egg albumin. Product control solution contained 0.05mL of each concentration of test samples and 0.45mL of distilled water, however test control was comprised of 0.45mL of BSA (0.5%) and 0.05mL of water (pH 6.3). Incubation was done at 37°C for 20 minutes. Then heated the test samples for 30 minutes at 57°C. Thereafter, samples were cooled and then added phosphate buffer (2.5mL) in each test tubes. Absorbance was determined using spectrophotometer at 660 nm (Qasim *et al.*, 2020).

$$\left[ \text{Inhibition (\%)} = 100 - \frac{\text{abs. of test solution} - \text{abs. of product control}}{\text{Abs. of test Control}} \times 100 \right]$$

#### HRBC membrane stabilization assay

Blood was assorted with equivalent size of Alsever's solution and was centrifuged for 15 minutes at 3000 rpm.

The supernatant liquid was poured out with help of micropipette and pack cells washed with isosaline solution (10%). Suspension of HRBC was prepared (10% v/v) with isosaline solution. The test solution was comprised of 1.0mL buffer solution, 0.5mL of RBC suspension, 2.0mL of hypotonic saline and 0.5mL of several concentrations 50-6400µg/mL of MA. Similar concentrations of aspirin as a standard were prepared respectively. Test control was comprised of 0.5mL of distill water instead of mandelic acid. Then test samples incubated at 37°C for 30 minutes in an incubator. Centrifuged the test samples at 3000 rpm for 5 minutes. Supernatant liquid was decanted out and optical density was measured at 560 nm by using UV-visible spectrophotometer. Percent protection against lysis was measured as follows (Qasim *et al.*, 2020).

$$\left[ \text{Inhibition (\%)} = 100 - \frac{\text{Abs. of test Samp}}{\text{Abs. of test Control}} \times 100 \right]$$

#### In-vivo formaldehyde induced arthritis in rat

This method was used to screen out the antiarthritic potential of mandelic acid against arthritis by formaldehyde in rats (Ahsan *et al.*, 2021). Rats were separated into several groups. Group-I (Arthritic control) group and received only distilled water (3mL/kg). Group-II received naproxen sodium (20mg/kg), Group-III, IV, V were given 40, 80, 160mg/kg of MA separately. On day one after 30 minutes of administration of doses, arthritis was induced by 0.1mL of sub-plantar injection of formaldehyde (2%) in left hind paw of rats. Induction was also carried out on day-3. Drug was administered continuously for 10 days orally. Paw volume was measured with the help of digital plethysmometer. The %age reduction in paw volume was equated using following formula.

$$\left[ \text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100 \right]$$

#### Acute toxicity

An acute toxicity study of mandelic acid was performed by following procedure of OECD guidelines 407 with minor modifications. A limit test was performed at dose level 2000mg/kg of MA to each rat (n=5). After 30 min of dosing, each animal was observed at least once and then periodically during the 1<sup>st</sup> 24 hour. Thereafter, daily for the period of 14 days for any clinical signs of toxicity and mortality (Nath and Yadav, 2015).

#### Sub-acute toxicity studies

Sub-acute toxicity study of mandelic acid was performed by following OECD guidelines with slight modifications. On the basis of above mentioned acute toxicity, three doses 200, 400 and 800mg/kg of MA were selected. Randomly Sprague Dawley rats were divided into 4 different clusters (n=6). Mandelic acid was administered daily to next three groups for a period of 14 days, while 1<sup>st</sup> group served as a control group received only distilled water.

During treatment all rats were kept under surveillance for any clinical signs and mortality for 14 days. Body weight was measured weekly. At the end of the treatment, after giving anesthesia to each rats blood was collected through cardiac puncture for evaluation of hematological and biochemical parameters. Heart, kidney and liver of rats were separated out and fixed in 10% formalin solutions. Paraffin section was stained with eosin and hematoxylin for detailed examination of histopathology of heart, liver and kidney (Nath and Yadav, 2015).

#### Sub-chronic toxicity studies

Sprague Dawley male and female rats were divided into four clusters (each group contains 5 females and 5 male rats). Group-I served as a control group and received only 3mL/kg distill water while group II, III and IV had 40, 80 and 160mg/kg of MA orally for the period of 28 days (OECD guidelines with slight modifications). At the end of the treatment, blood was collected in EDTA tubes and non-heparinized tubes immediately for the estimation of hematological and biochemical parameters. Kidney, heart and liver of rats were removed and rinsed in 0.9% NS and then weighed. After this, these organs were fixed in 10% formalin solution and stained with eosins and hematoxylin for histopathological examination of these organs with control group (Zakaria *et al.*, 2016).

#### STATISTICAL ANALYSIS

Values were represented as mean  $\pm$  standard error mean. Statistical scrutiny were carried out by 2-way ANOVA, followed by Bonferroni posttest using Graph pad Prism (Version 5.01) and ONE way ANOVA followed by Dunnett test. Level of confidence was considered as \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$  and <sup>ns</sup> non-significant.

#### RESULTS

##### Effect of Mandelic acid on egg albumin protein denaturation

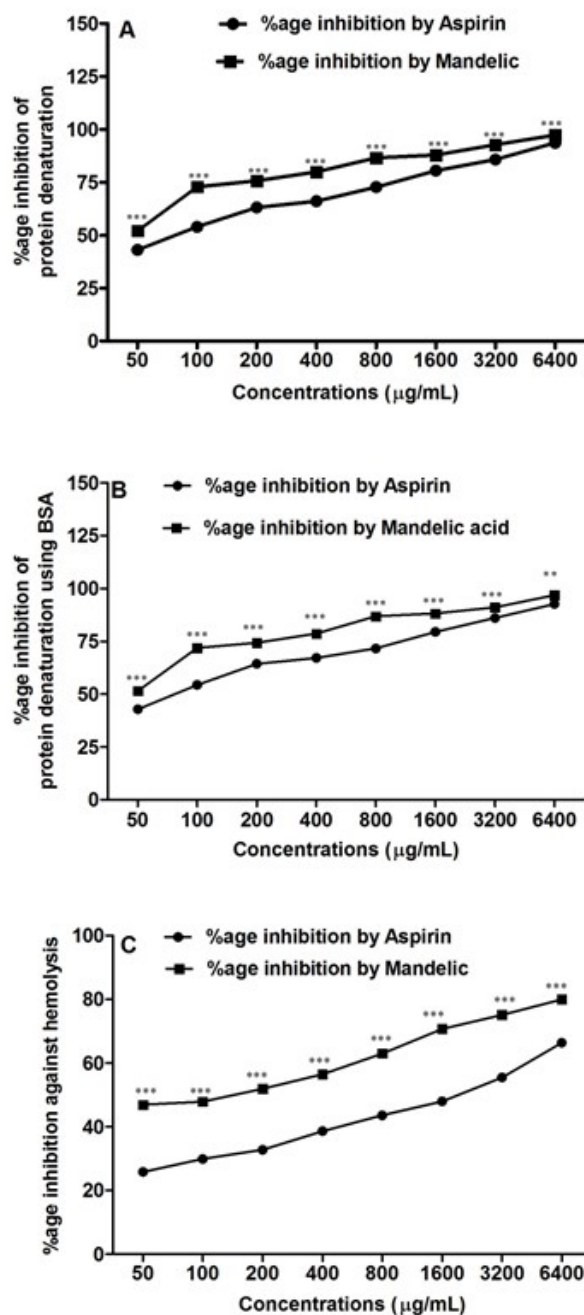
Mandelic acid showed gradual increase in prevention of protein denaturation. The major increase (28.60%) was observed when we doubled the dose from 50 to 100 $\mu\text{g/mL}$  (fig. 1A). Later on 5-7% rise in inhibition of protein denaturation was noted at all higher doses. Mandelic acid exhibited highest inhibition (97.29%) at 6400 $\mu\text{g/mL}$  when compared with aspirin (93.63%).

##### Effect of Mandelic acid on thermally induced BSA protein denaturation

The results on inhibition of thermally induced (BSA) protein denaturation was almost same as on egg albumin thermally induced protein denaturation at initial concentration. fig. 1B also reveals that mandelic acid possessed highest percent protection (96.90%) against thermally induced denaturation at 6400  $\mu\text{g/mL}$ , which was greater than percent inhibition by aspirin (92.62%) at same concentration.

##### Effect of Mandelic acid on HRBCs membrane stabilization assay

In this method, MA protected concentration dependently the membrane of erythrocytes from hemolysis. MA exhibited greater fortification of HRBC membrane (80.03%) in contrast to hypotonicity persuaded lysis at 6400 $\mu\text{g/mL}$  as compared to aspirin (66.36%) as depicted in fig. 1C.



**Fig. 1(A-C):** Effect of percentage inhibition of Mandelic acid on protein denaturation and HRBCs membrane stabilization assay. Values represented as Mean  $\pm$  SEM, followed by Bonferroni Posttest using 2-way ANOVA, \*\*\* ( $p < 0.001$ ) vs standard (Aspirin).

**Table 1:** Effect of multiple oral doses of mandelic acid for 10 days on formaldehyde induced paw volume

Treatment groups	2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day
Increase in paw volume					
Arthritic control	0.64±0.06	0.818±0.074	0.882±0.078	0.912±0.102	0.938±0.23
Naproxen sodium (20 mg/kg)	0.364±.108* (43.827%)	0.314±0.038*** (61.613%)	0.324±0.019*** (63.265%)	0.282±0.026*** (69.078%)	0.274±0.02*** (70.788%)
Mandelic acid (40 mg/kg)	0.408±0.057 <sup>ns</sup> (37.037%)	0.434±0.042*** (46.943%)	0.442±0.030*** (49.886%)	0.452±0.031*** (50.438%)	0.438±0.06*** (53.304%)
Mandelic acid (80 mg/kg)	0.340±0.068* (47.530%)	0.394±0.015*** (51.833%)	0.346±0.015*** (60.770%)	0.324±0.029*** (64.473%)	0.286±0.019*** (69.509%)
Mandelic acid (160 mg/kg)	0.338±0.039* (47.839%)	0.352±0.039*** (56.968%)	0.360±0.060*** (59.184%)	0.302±0.042*** (66.88%)	0.212±0.024*** (77.398%)

Values are expressed as mean ± SEM (n=5) done by Two way ANOVA followed by boneferonnie posttest as compared to arthritic control group. \*p<0.05, \*\*\*p<0.001.

**Table 2:** Biochemical and hematological parameters analysis in sub-acute toxicity studies after oral administration of mandelic acid for 14 days

Biochemical and Blood parameters	Normal control	Mandelic acid (200 mg)	Mandelic acid (400 mg)	Mandelic acid (800 mg)
ALT (U/I)	39.29 ± 0.35	36.52 ± 0.83**	38.27 ± 0.38***	85.58 ± 7.49***
AST (U/I)	44.22 ± 0.66	48.85 ± 0.61 <sup>ns</sup>	49.99 ± 0.00 <sup>ns</sup>	87.95 ± 7.23***
ALP (U/I)	181.77± 1.11	190.60 ± 0.30 <sup>ns</sup>	196.97 ± 2.59 <sup>ns</sup>	227.21 ± 0.94***
Total protein (g/dL)	7.23 ± 0.04	5.96 ± 0.40**	7.13 ± 0.05 <sup>ns</sup>	5.46 ± 0.12**
Urea (mg/dL)	26.10 ± 0.34	29.80 ± 0.09*	33.03 ± 0.61***	53.43 ± 1.12***
Creatinine (mg/dL)	0.93 ± 0.02	0.81 ± 0.05 <sup>ns</sup>	0.81 ± 0.02 <sup>ns</sup>	0.98 ± 0.01 <sup>ns</sup>
Cholesterol (mg/dL)	79.58 ± 4.97	89.21 ± 5.04 <sup>ns</sup>	79.59 ± 0.35 <sup>ns</sup>	112.33 ± 1.45***
Triglycerides (mg/dL)	85.40 ± 1.41	87.60 ± 1.24 <sup>ns</sup>	91.73 ± 0.37*	109.21 ± 1.30***
Blood sugar (random)	98.44 ± 0.24	107.50 ± 0.68***	105.33 ± 0.27***	111.39 ± 0.40***

**Table 3:** Effects on biochemistry in sub-chronic toxicity studies with different doses of mandelic acid for 28 days

Biochemical and Blood parameters	Normal control (3 mL/kg)	Mandelic acid (40 mg/kg)	Mandelic acid (80 mg/kg)	Mandelic acid (160 mg/kg)
ALT (U/I)	86.44 ± 0.39	88.96 ± 0.52 <sup>ns</sup>	81.12 ± 1.10**	81.35 ± 0.63**
AST (U/I)	47.42 ± 0.39	43.83 ± 0.89*	49.22 ± 0.38 <sup>ns</sup>	51.86 ± 0.90**
ALP (U/I)	236.11± 4.57	268.74 ± 4.02***	248.37 ± 1.48 <sup>ns</sup>	241.47 ± 0.71 <sup>ns</sup>
Total protein (g/dL)	7.48 ± 0.06	4.98 ± 0.05 <sup>ns</sup>	6.96 ± 0.04 <sup>ns</sup>	5.16 ± 1.98 <sup>ns</sup>
Urea (mg/dL)	30.21 ± 0.11	27.05 ± 0.35***	22.08 ± 0.41***	24.06 ± 0.29**
Creatinine (mg/dL)	0.84 ± 0.02	0.75 ± 0.04 <sup>ns</sup>	0.75 ± 0.02 <sup>ns</sup>	0.67 ± 0.00*
Cholesterol (mg/dL)	95.95 ± 1.64	87.14 ± 0.74***	90.70 ± 0.37*	90.63 ± 0.99**
Triglycerides (mg/dL)	96.55 ± 0.66	82.52 ± 0.26***	85.80 ± 0.13***	89.30 ± 0.34***
Blood sugar (random)	101.00 ± 0.56	108.95 ± 0.63**	112.65 ± 1.60***	118.07 ± 1.36***

Data are expressed as mean ± SEM. Results were compared with normal control group. One-way ANOVA was followed by Dunnet test.

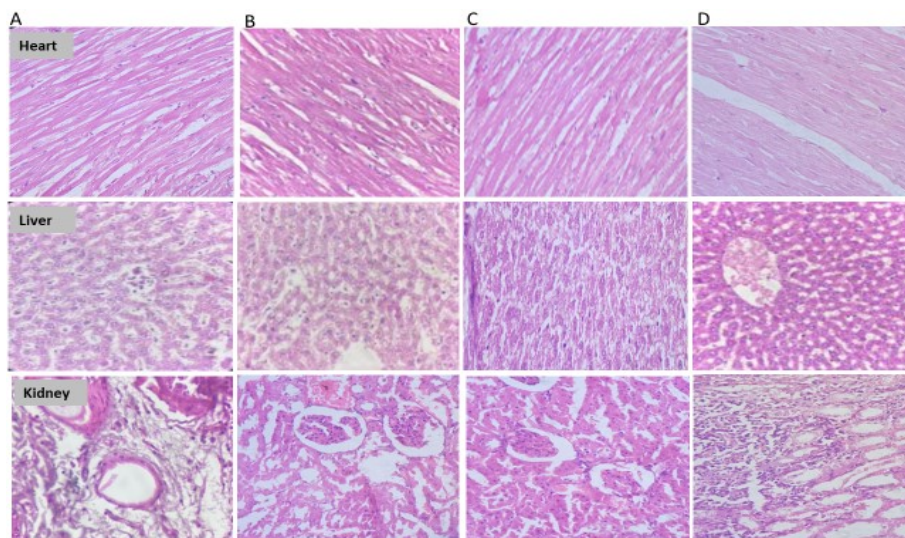
**Effect of Mandelic acid on non - immunological arthritis induced by formaldehyde**

Anti-arthritic effect of mandelic acid was also appraised by using *in-vivo* rat model of arthritis. Results were equated with arthritic control animals, which presented remarkable escalation in paw volume (0.938±0.23) throughout the study period. It was noted that on 2<sup>nd</sup> day of study, maximal reduction in paw volume was observed as 47.53% and 47.83% at 80 and 160mg/kg doses respectively, while at 10<sup>th</sup> day dose dependent reduction was detected. The evaluation made on day 10 presented that treatment with MA noticeably (p<0.001) dwindled injected paw volume at 40, 80 and 160mg/kg i.e., 53.30,

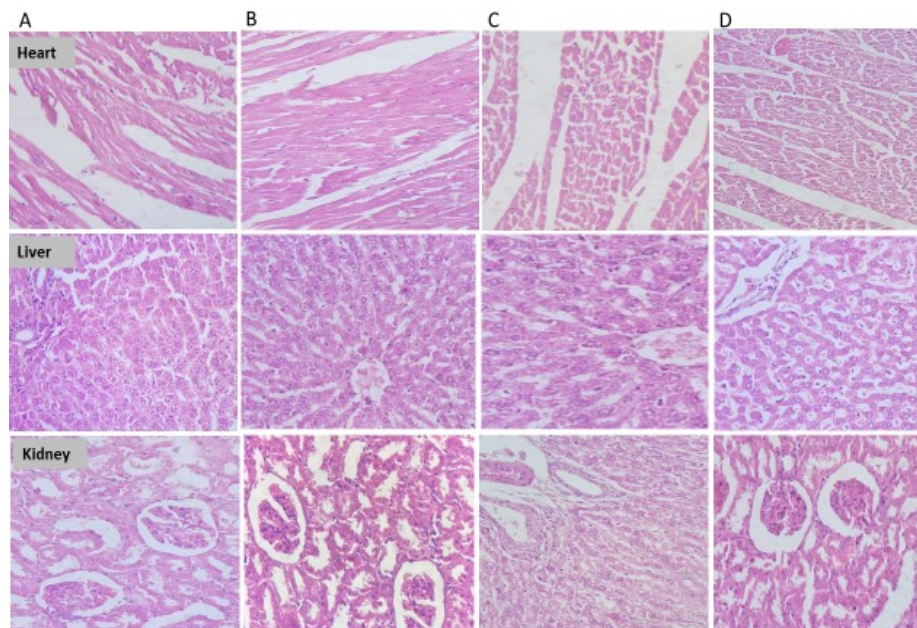
69.51 and 77.40%, correspondingly as compared with non-treated rheumatic control group (table 1).

**Acute toxicity study of mandelic acid**

Data from current investigations revealed that single dose oral administration of MA 2000mg/kg did not exhibited significant changes in general behavior like autonomic response (salivation, urination, lacrimation and myopia) and cutaneous changes (skin itching and irritation, changes in fur). Meanwhile, any sign of toxicity and mortality did not displayed in each rats during observation period of 14 days.



**Fig. 2:** Histopathological examination of heart, liver and kidney in sub-acute toxicity study of mandelic acid for 14-days. A represents tissue slides of heart, liver and kidney of normal rats, B, C and D indicates tissue slides treated with MA 200mg/kg, 400mg/kg and 800mg/kg respectively



**Fig. 3:** Histopathological investigation of heart, liver and kidney in sub-chronic toxicity study of mandelic acid for 28-days. A represents slides of heart, liver and kidney of normal rats, B, C and D indicate slides of heart, liver and kidney treated with MA 40mg/kg, 80mg/kg and 160mg/kg.

#### ***Sub-acute toxicity of mandelic acid***

In sub-acute treatment oral administration of 200,400 and 800mg/kg MA did not presented any visual sign of toxicity and mortality to rats during entire period. Furthermore, biochemistry examination of rats that received 200 and 400mg/kg, did not exhibit any noticeable adverse effects but biochemical test of rats treated with 800mg/kg showed slight increase in AST, ALP and urea (table 2). Mandelic acid showed no significant divergence in organ weight (kidney, liver and heart) at different doses 200, 400 and 800mg/kg when compared with control rats. In histopathological

assessment of kidney, heart and liver showed a normal organization of hepatocytes around liver with normal myofibril arrangements in heart tissues and normal distal convoluted tubules in kidneys in rats treated with 200 and 400 mg/kg while minor inflammatory cells are visible with slight dilated sinusoids in rats treated with 800mg/kg MA (fig. 2).

#### ***Sub-chronic toxicity of mandelic acid***

Oral administration of mandelic acid at 40, 80 and 160 mg/kg to rats for the period of 28 days did not produced any considerable alterations in weight of vital organs on

the time of dissection as well as weekly measurement of body weight throughout the treatment. There was no variations found in biochemical and hematological blood parameters in rats treated with MA as compared to normal control rats (table 3). There was no significant adverse effect presented in microscopic examination of histopathology of heart, liver and kidney. All slides presented normal arrangement of cardiac tissues, hepatocytes and glomerulus of kidney as shown in fig. 3.

## DISCUSSION

Findings from above study showed that denaturation of protein is the leading source of inflammatory illnesses that is responsible for the release of autoantigens, thus initiates the release of various antigens that result in arthritic disorder. It has been notified that any alteration in disulphide, hydrogen and electrostatic bond is a promising mechanism involved in protein denaturation (Precupas *et al.*, 2016). Therapeutic products that have ability to inhibit the protein denaturation would be appreciated for improvements against arthritis. In denaturation process, state of protein changed from soluble to insoluble form due to presence of chemical agents like acids, dyes, alkalies, heavy metals. Besides that, denaturation is a reaction between water and protein that leads towards hydrolysis (Sangeetha and Vidhya, 2016). Flavonoid and phenolic compounds are the secondary metabolites of plants and possess various biological activities like antiaging, anti-atherosclerosis, anti-inflammatory, protection against cardiovascular damage and prevents the cell proliferation and angiogenesis of endothelial function (Rahman *et al.*, 2015). In present analysis, mandelic acid prevented denaturation and its effect was significantly higher than aspirin. On the basis of aforementioned outcomes, so we can say that mandelic acid reduces the liberation of auto-antigens, oxygen free radicals and pro-inflammatory mediators resulting in inhibition of protein denaturation, which is favorable in dismissing the pain related with arthritis.

Stabilization of HRBC membrane against lysis proposes additional mechanism of antiarthritic drugs induced by hypotonic solution. Hypotonic solution causes the accumulation of fluid which is important for shattering of lysosomal membrane and causes the liberation of phospholipase (A2) and release of inflammatory cells. Hereafter, lysosomal membrane stabilization inhibits the release of provocative contents, thus, decreases the rupturing of cells and inhibits the tissue damage (Umukoro and Ashorobi, 2006). They inhibit the liberation of lysosomal content at inflammation site from neutrophils (Sangeetha and Vidhya, 2016). Mandelic acid inhibited lysis of membrane induced by hypotonic solution in a dose dependent fashion, when compared with aspirin. These findings suggest that membrane stabilization serve as an additional mechanism of

mandelic acid against arthritis, this is may be due to reaction of membrane components with phytoconstituents of mandelic acid, and however precise mechanism of stabilization is not known.

Arthritis induced by formaldehyde is one of the most important model for the screening of new agents with anti-arthritic and anti-inflammatory activity because it shares the close similarity to human arthritis (Ben *et al.*, 2016). Formaldehyde at injection site causes protein denaturation which leads to progression of reaction in contrast to degraded products (Nair *et al.*, 2012). Additionally, formaldehyde persuaded inflammation is biphasic in nature and neurogenic component initiates the cascade of cell mediated reactions. They reduce the paw volume by decreasing influx of inflammatory debris. Mandelic acid possess the highest effect at 160mg/kg and it acts centrally and inhibits both phases of inflammation.

Phytochemicals are novel therapeutic agents obtained from various plants and possess pharmacological and biological activities. In addition structure activity relationship showed that hydroxyl structures are dominant for antioxidant activity because they increase the scavenging and reducing ability of the compound (Gordon and Roedig-Penman, 1998). Anti-rheumatic consequences of MA might be due to the presence of hydroxyl group in it. As MA comprises of 2 major targets, like, hydroxyl group which have ability to donate electron, 2<sup>nd</sup> presence of phenyl group and acetic acid that might be able to inhibit the COX-2 enzymes as was discussed in synthesis and SAR of diclofenac analogue, which also possess phenyl acetate group in its structure (Gordon and Roedig-Penman, 1998; Moser *et al.*, 1990).

In present investigation acute treatments to five rats did not revealed any sign of toxicity and mortality after single exposure of MA (2000mg/kg) at different intervals during observation period of 14 days. According to labeling of chemicals and globally harmonized system of classification, substances having LD<sub>50</sub> higher than 2000 mg/kg are designated as relatively safe (Konan *et al.*, 2007). No sign of toxicity (adverse effects) and mortality was observed during entire period in groups treated with 200 and 400mg/kg. While hematological and biochemical test's evaluation showed that there was a slight increase in level of AST, ALT, ALP and urea in rats treated with 800 mg/kg of mandelic acid. Alanine aminotransferase is a cytoplasmic enzyme present in liver, increase in this enzyme suggested the hepatocellular damage of liver in rats. While significant rise in urea showed adverse effects in kidney function of rats at high doses (Nath and Yadav, 2015). In histopathological assessments, microanatomy of heart, kidney and liver did not presents any treatment related adverse effect at doses with 200 and 400mg/kg of mandelic acid. However histological examination of heart, kidney and liver of rats received 800mg/kg exhibited slight adverse effects like disorganization of

glomerulus and tubules, irregular arrangements of dilated sinusoids in the liver. Furthermore, MA did not affect the body weight of rats during both Sub-acute and Sub-chronic toxicity studies. Thus they do not influence the food consumption and general behavior of rats treated with mandelic acid. In sub-chronic toxicity studies, no anomalous alterations were found in biochemical and hematological parameters.

## CONCLUSION

Hence, it can be suggested that mandelic acid can be an alternative agent for treatment of rheumatoid arthritis. Anti-arthritis effect may be due to stabilization of free radicals produced at site of inflammation and inhibiting the production of inflammatory mediators. Furthermore, the toxicity outcomes ensure its safety at therapeutic doses and revealed no considerable changes in cardiac muscles, nephrons and hepatic portal vein of liver.

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