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Histopathological Evaluation of Gastro-Hepatoprotective Effect of *Cassia fistula* versus Naproxen in Rat Model of Rheumatoid Arthritis

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a debilitating disease leading to incapacitation and deformity due to side effects of anti arthritic drugs and chronic need to use these drugs, patients often switch to and prefer phytotherapy. **Aims & Objectives:** The present study was the histopathological evaluation of gastro-hepatoprotective effect of *Cassia fistula* compared to naproxen in rheumatoid arthritis murine model. **Place and duration of study:** This three month study was conducted at the Animal House, University of Veterinary and Animal Sciences, Lahore. **Material & Methods:** Ninety six male rats were randomly sorted into 12 groups (n=8). CFA injection (0.2 ml) was administered in the right hind paw of all groups except Group 1 (negative control). Group 2 (positive control) and groups 3-7 (prophylactic groups) were administered orally BD on 3 consecutive days 1,2 &3, naproxen (25mg/kg), anthraquinone (250mg/kg and 500mg/kg) and methanolic (250mg/kg and 500mg/kg) extracts of *Cassia fistula* respectively, the first dose given 30min prior to CFA injection .Groups 8-12 (therapeutic groups) were given naproxen, anthraquinone and methanolic extracts orally BD respectively in the same doses on 3 consecutive days 9, 10 and 11. On Day 15, histopathology of stomach & liver was done. **Results:** Oral anthraquinone and methanolic extracts of *Cassia fistula* 500mg/kg vs 250mg/kg BD, in prophylactic groups normalized histology of gastric and liver tissue more as compared to the therapeutic groups and to naproxen gastrohepatic damage . **Conclusion:** Cassia fistula anthraquinone and methanolic extracts exhibited greater prophylactic than therapeutic, dose dependent gastrohepatoprotection as compared to naproxen damage to these organs in the CFA rheumatoid arthritis rat model.

Key words: *Cassia fistula*, Complete Freund's Adjuvant (CFA), naproxen, gastrohepatoprotective

INTRODUCTION

Rheumatoid arthritis is a systemic autoimmune pathology mainly characterized by synovitis and joint destruction leading to incapacitation and disability[1]. It is associated with multiorgan dysfunction such as splenomegaly, lung fibrosis, pleural effusion, amyloidosis, muscle wasting, thinning of skin, peripheral neuropathy, hepatic and renal dysfunction. Cytokines play a key role in the perpetuation of synovial inflammation[2].

Naproxen, a nonspecific NSAID is chiefly used to diminish pain and swelling of RA[3] but it is highly nephrotoxic, gastro and hepatotoxic. It can elicit various types of injury including acute renal damage, acid–base imbalance, ulceration, and perforation of the stomach or intestines, melena, and hematemesis, hepatocellular to cholestatic injury, acute liver failure and even death[4].

Natural substances have innumerable medicinal properties and can cure without any adverse effects. *Cassia fistula*, a popular ornamental plant is of great importance in treating arthritis along with its gastroprotective and hepatoprotective potentials[5].

All parts of this plant have been used in traditional herbal medicines however its fruit pulp has been found effective in gout and rheumatoid arthritis and possesses anti-inflammatory, gastroprotective, hepatoprotective and nephroprotective activity.[6] Its bark exhibited the highest antioxidant potential. Natural antioxidants played an important role in prophylaxis of the chronic and degenerative illnesses such as rheumatic disorder[7].

The current study was to compare the gastric and hepatoprotective ability Cassia Fistula extracts against gastric and hepatic damage caused by naproxen while treating rheumatoid arthritis model. Our study was also unique because the effect of prophylactic and therapeutic administration of these extracts had not been determined employing histopathology in this model.[8,9]

MATERIAL AND METHODS

96 male wistar albino rats weighing 170–200 gms were housed and acclimatized for a week at the UVAS Animal House under standard housing temperature, humidity conditions and dietary specifications.[14]

Pulp of fruit and bark of *Cassia fistula* were gathered from Botany Department of Punjab University, Lahore.

Development of rat model of rheumatoid arthritis: To elicit joint injury in right hind paw of rats, 0.2 ml of CFA was injected.[13,14] A gradual increase in swelling was observed surrounding the injection site along with increasing immobility due to arthritis over 9th to 15th post CFA injection day period. Treatment was initiated on day 1 (prophylactic model) and day 9 (therapeutic model).

Extraction of anthraquinone:

The extraction process was performed at PCSIR.[14] 30gm of pulverized cassia fruit pulp were added to 150ml of ethanol in a 1:5 concentration in Soxhlet apparatus and extracted over 24hrs. Anthraquinone (rhein) extract was concentrated, dried and stored with desiccant.[10,11].

Preparation of methanolic extract:

The dessicated and pulverized bark was processed to remove fats and extracted using methanolic distillation in a soxhlet extractor[14]. The extract was further dried using a rotary vacuum evaporator[12]. 9% w/w Cassia fistula methanolic extract was obtained.

Experimental setup:

Ninety six male rats were grouped into 12 (n=8).Test extracts and standard drug water suspensions were made employing 1% carboxymethyl cellulose as suspending agent.

Group 1 (Healthy Control): comprised of healthy male rats.

Group 2 (Positive Control): Rats were induced with rheumatoid arthritis by Complete Freund's Adjuvant (CFA) and left for natural recovery.

Prophylactic Groups 3-7 (drugs administered orally BD on days 1, 2 & 3. With the 1st dose given 30 min before CFA injection)

Group 3: Naproxen 25mg/kg.
Group 4: anthraquinone250mg/kg.
Group 5: anthraquinone 500mg/kg.
Group 6: methanolic extract 250mg/kg.
Group 7: methanolic extract 500mg/kg.

Therapeutic Groups 8-12 (Drugs administered orally BD for 3 days on Day 9, 10 & 11 following CFA Injection)

Group 8: naproxen 25mg/kg
Group 9: anthraquinone extract at dose 250mg/kg.
Group 10: anthraquinone extract at dose 500mg/kg
Group 11: methanolic extract at dose 250mg/kg
Group 12: methanolic extract at dose 500mg/kg.

Histopathological Studies of viscera:

At the end of study the rats were sacrificed .The stomach and liver kidney tissues were removed, ice cold saline washed and fixed in 10% formalin solution. Hematoxylin and eosin tissue staining was employed.

The histopathology was evaluated using following grading: Mild(+), Moderate(++), and Intense(+++).

Grading of Stomach damage:

Mild: Cellular debris(<25 %), Intact mucosa, Intact muscularis propria, Intact gastric pits and gastric glands

Moderate: Cellular debris(26-50 %), Ulcerative gastric mucosa

Intense: Cellular debris(>50 %), Ulcerative gastric mucosa, fibrinoid necrosis with granulation tissue

Grading of Liver damage:

Mild: Epithelium intact, Nuclear pyknosis (<25 %), No fatty infiltration, No centrilobular necrosis.

Moderate: Epithelium not fully intact
Nuclear pyknosis (26-50%), No fatty infiltration, Centrilobular necrosis seen.

Intense: Epithelium not intact, Inflammatory infiltrate exhibited hepatic damage with, Nuclear pyknosis (>50%), Centrilobular necrosis with fatty infiltration

Statistical analysis:

Only representative slides of gastric and hepatic tissue gathered during this research are presented here with no statistically significant association.

RESULTS

Stomach:

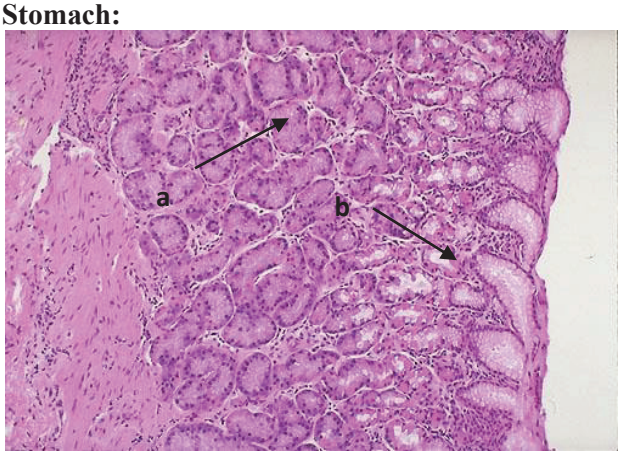

Fig-1: Gastric histopathology of healthy control (group 1) showed (a) intact submucosa and (b) muscularis propria (40X).

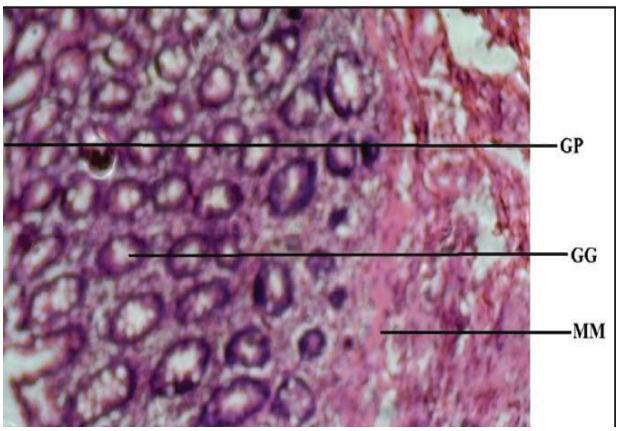

Fig-4: Histology of rat stomach showing gastric gland (GG), gastric pit (GP) and muscularis mucosa (MM). H & E stain having therapeutic Anthraquinone 250mg/kg with normalized gastric mucosa (100X)

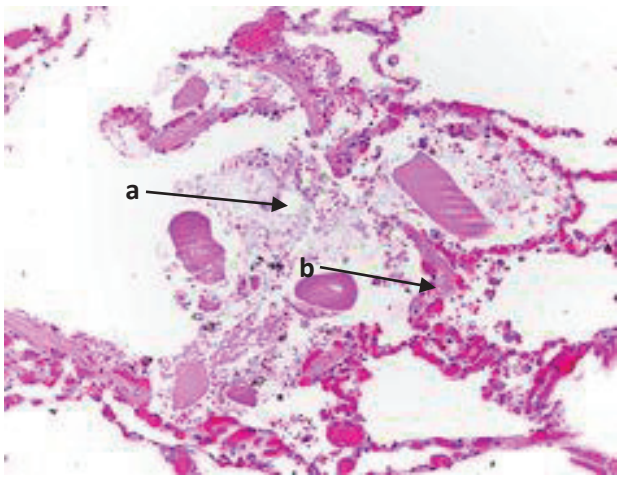

Fig-2: Gastric histopathology of rats treated with naproxen prophylactically showed (a) ulceration of gastric mucosa with (b) layers of inflammation and necrosis(40X).

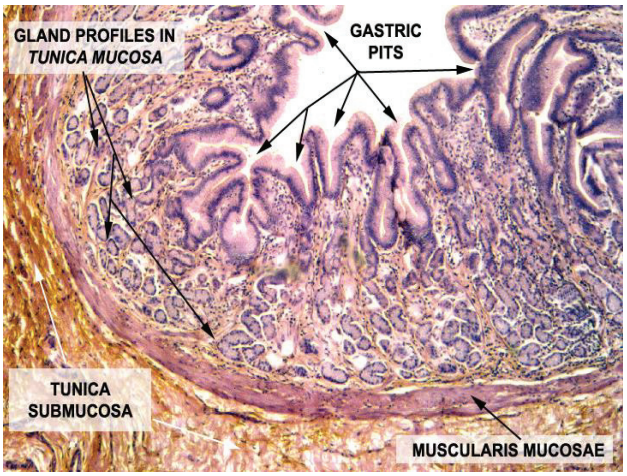

Fig-5: Histopathology of Group 12 in which methanolic extract 500mg/kg was administered therapeutically.(100X)

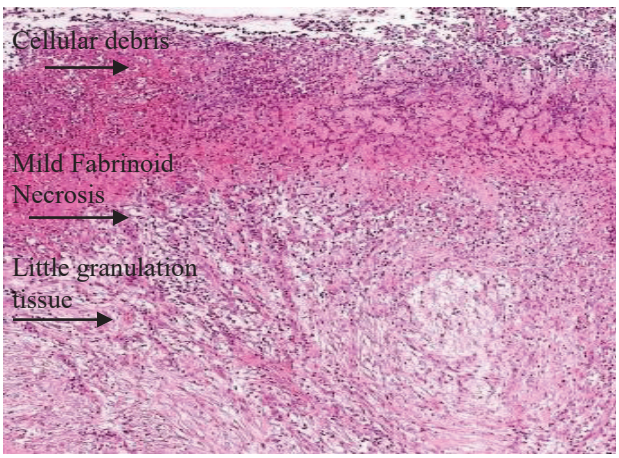

Fig-3: Gastric tissue also showed mild gastric ulcers in naproxen treatment (40X).

Liver:

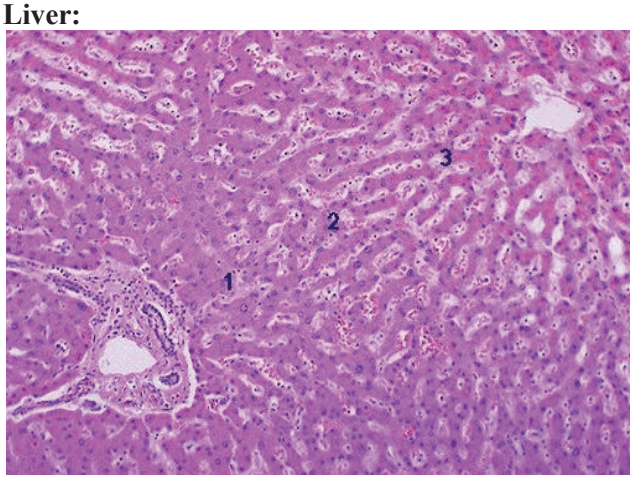

Fig-6: Shows normal architecture and intact epithelium of liver. (1.Central Vein, 2.Portal Triad, 3.Three Zones of Liver Lobule) (40X).

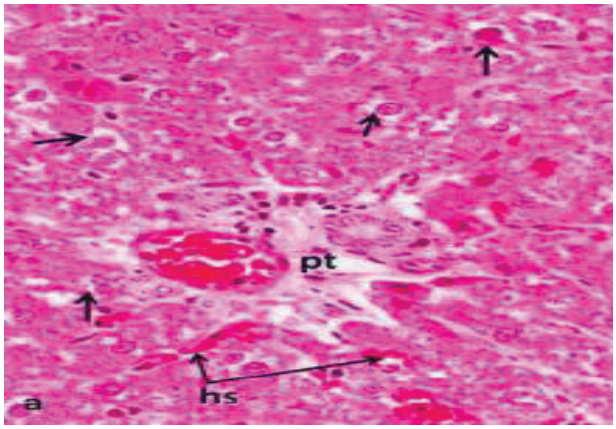

Fig-7: Shows vacuolated hepatocytes in the periportal zone due to prophylactic naproxen induced damage. Pt=Portal triad, hs=Hepatic sinusoid. (40X)

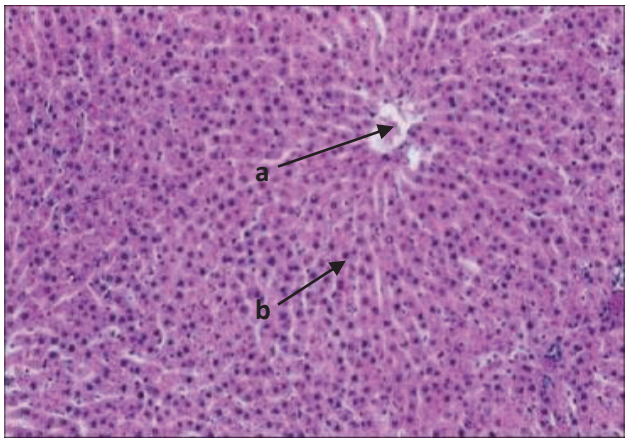

Fig-8: Arrows shows (**a)** central vein, (**b)** intact epithelium, normal hepatic architecture & absence of nuclear pyknosis, fatty infiltration and centrilobular necrosis of liver in rats prophylactically treated with 250mg/kg anthraquinone extract of cassia fistula. (40X)

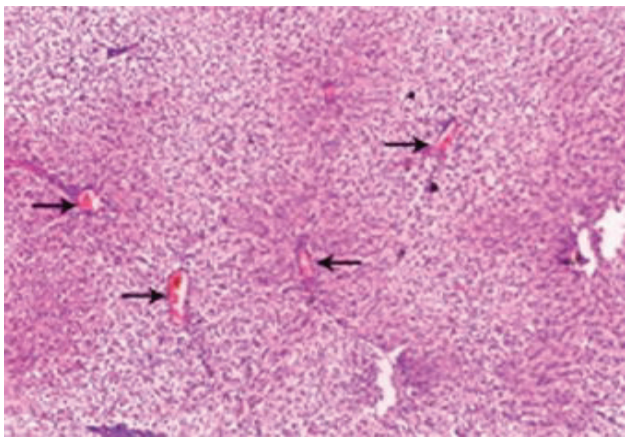

Fig-9: Shows no nuclear pyknosis and absence of fatty infiltration and centrilobular necrosis in rats therapeutically treated with 500mg/kg methanolic extract of cassia fistula. Epithelium was intact. The hepatic lobules show central veins (arrows) from which the hepatocytes radiate in the form of cords. These cords are separated by blood sinusoids (40X)

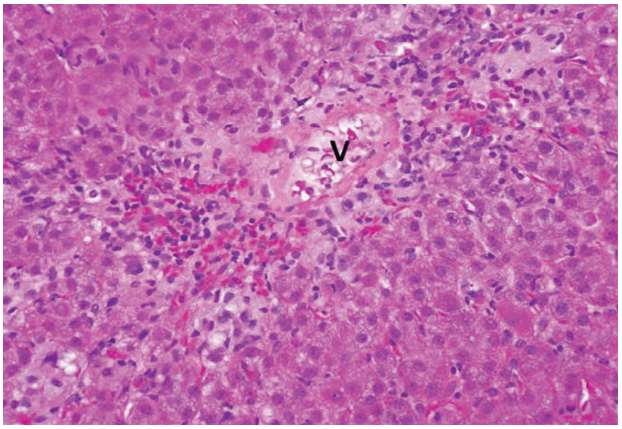

Fig-10: Shows liver hepatotoxicity after therapeutic naproxen administration. Hepatocytes around the central vein have been lost and replaced by an inflammatory infiltrate composed of lymphocytes and plasma cells, exhibited hepatic damage with nuclear pyknosis and centrilobular necrosis. (40X)

DISCUSSION

RA is an inflammatory autoimmune disease affecting joints and multiple systems of the body represented by chronic synovitis, generalized inflammation, and autoantibodies (particularly to rheumatoid factor). Naproxen is an NSAID used to treat RA but has various adverse effects.[15]

Research showed that Cassia fistula given both prophylactically and therapeutically exhibited anti-arthritic effects.[16] Our present study was conducted on Cassia fistula bark and fruit pulp to evaluate its gastro-hepatoprotective histological effect in CFA induced RA model. CFA administration itself did not produce any gastric or hepatic pathology.

Gastric Histopathology:

Gastric histopathology of healthy control group 1 was normal at the end of experiment. (Fig-1)

Group 3 in which naproxen 25mg/kg BD was administered, showed gastro-ulcerative effects including cellular debris, ulcerative gastric mucosa and fibriniod necrosis with formation of granulation tissue (Fig-2&3). These inflammatory changes were due to the inhibition of prostaglandin production and alsoshowed moderate gastric damage due to the production of reactive oxygen species. .

250mg/kg BD anthraquinone and methanolic extracts of cassia fistula given either prophylactically or therapeutically, maintained normal gastric tissue(Fig- 4).

Doubling the dose of anthraquinone and methanolic extracts to 500mg/kg BD resulted in normalization of mucosa and muscularis propria with intact gastric glands(Fig- 5) almost similar to histology of healthy control group 1 (Fig-1)

The dose dependent improvement in gastric histology is the antiulcerogenic result of hindrance

in 5 lipoxygenase intervening in lipid peroxidation of arachidonic acid and free radical induced lipid peroxidation, hence inflammatory changes were prevented.[16]

Hepatic histopathology:

Hepatic histopathology of healthy control group 1 was normal at the end of experiment (Fig-6).

The naproxen treated groups 3(prophylactic)&8 (therapeutic) , showed hepatocellular liver damage with 25mg/kg BD dose (Fig-7 & Fig-10) with inflammatory infiltration, nuclear pyknosis associated with centrilobular necrosis and fatty infiltration because prostaglandin production was retarded. Injury was also due to excessive production of reactive oxygen species and reduction in major antioxidant levels.

On administration of anthraquinone and methanolic extracts of cassia fistula 250mg/kg BD both prophylactically(Fig-8) and therapeutically, an improvement in liver histology was noted as moderate changes i-e epithelium not fully intact, lesser nuclear pyknosis (26-50%) and no fatty infiltration.

On the other hand, anthraquinone and methanolic extracts of cassia fistula in higher 500mg/kg doses BD in both prophylactic and therapeutic groups prevented any hepatic damage. Revealed by an intact epithelium,<25% nuclear pyknosis with no fatty infiltration and centrilobular necrosis.(Fig-9)

The underlying of the hepatoprotective mechanism could be the retardation lipoxygenase mediated lipid peroxidation of arachidonic acid and free radical induced lipid peroxidation, hence leukotriene biosynthesis and chemotaxis was decreased.[16,17]

CONCLUSION

Cassia fistula anthraquinone and methanolic extracts exhibited greater prophylactic than therapeutic, dose dependent gastrohepatoprotection as compared to naproxen damage to these organs in the CFA rheumatoid arthritis rat model.

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