ORIGINAL ARTICLE

Comparing Hepatoprotective Effects of Aqueous Extract of Cassia Fistula (Amaltas) Leaves and Silymarin

Rukhsana Munawar, Adnan Jehangir, Akbar Waheed

ABSTRACT

Objective: To compare the hepatoprotective effects of aqueous extract of *Cassia fistula* leaves and Silymarin on Acetaminophen induced hepatotoxicity.

Study Design: An experimental study in mice.

Place and Duration of Study: The study was conducted in the department of Pharmacology and Therapeutics, Multidisciplinary research laboratory of Islamic International Medical College, Riphah International University. The duration of study was from 16 Mar, 2016 to 16 Mar, 2017.

Materials and Methods: In present study simple random sampling was done by Balloting method and sixty adult Balb/c Albino mice were divided in four groups having 15 mice each. Mice in control group A were given normal diet and fresh water. Mice in experimental group B (positive control) were given Acetaminophenat dose of 100mg/kg/day orally for six weeks. Mice in experimental group C were treated with Acetaminophen(100mg/Kg) with concurrent 400mg/Kg body weight of *Cassia fistula* (Aqueous extract) orally for six weeks once daily while in experimental group D mice received 750 mg/kg body weight of Silymarin orally in form of aqueous suspension in 2% Cremophor1E for 6 weeks once daily concurrently treated with Acetaminophen(100mg/Kg).The cardiac puncture was done after six weeks and 2.5cc blood was collected from each mice for assessment of biochemical markers including Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST).

Results: The mean value of serum ALT level was 130.4.30 ±42.90 in Group B as compared to control Group having mean value of ALT 37.30 ± 8.32. The mean value of ALT was 62.22± 19.27 and 53.90± 25.06 in Group C and in Group D respectively. In Group B mean value of serum level of AST was 134.3± 15.56 as compared to control Group A having mean value of AST31.40± 10.05.The mean value of AST in Group C and Group D were 66.40±20.50 and 44.67± 20.37 respectively.

Conclusion: The Silymarin is more beneficial than aqueous extract of *Cassia fistula* leaves in ameliorating hepatotoxic effects of Acetaminophen.

Key Words: *Acetaminophen, Cassia fistula leaves, Hepatoprotective, Silymarin.*

Introduction

Liver is a vital organ having role in metabolism of carbohydrates, proteins and lipids, bile production, maintenance of homeostasis, detoxification and vitamin storage.[1,2] Drug induced liver injury (DILI) is the leading cause of acute liver failure in most countries.[3] There are more than 1000 drugs considered toxic to liver. The incidence of DILI is 1 in 10,000 to 1 in 100,000.[4] In humans the most common cause of drug induced liver failure is acetaminophen (Paracetamol, N-acetyl-p-aminophenol,APAP) toxicity.[5] It is safe at therapeutic doses but at larger doses it can cause serious liver injury.[6,7] The cytochrome P-450 oxidation of acetaminophen overdose result in formation of reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which initiates toxicity.[8] It depletes glutathione and binds to cellular proteins, especially in mitochondria and cause hepatotoxicity.[7,9,11] Previous studies have also identified NAPQI as important factor in acetaminophen induced hepatotoxicity.[8] Previous studies have identified some hepatoprotective agents against acetaminophen induced hepatotoxicity. Silymarin

Department of Pharmacology
Islamic International Medical College
Riphah International University, Islamabad

Correspondence:
Dr. Rukhsana Munawar
Department of Pharmacology
Islamic International Medical College
Riphah International University, Islamabad
E-mail: dr.rukhsana33@gmail.com

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(SLM) is one of them having antioxidant, anti-inflammatory, antifibrotic, antiviral and immunomodulatory actions.[1,12,14] The Silymarin has hepatoprotective and antioxidant role by inhibiting free radicals.[1,15]

Herbal medicines represent one of the most important fields of traditional medicine throughout the world. *Cassia fistula* (Family Leguminosae, Sub-family Caesalpinea) is a very common plant known for its medical properties. It is commonly called Amaltas. The antioxidant property is possessed by polyphenol and flavonoids.[16]

Previous studies showed work done on methanolic and ethanolic extracts of *Cassia fistula* leaves. In present study aqueous extract was used to explore hepatoprotective effect of *Cassia fistula* leaves and its hepatoprotective effect was compared with Silymarin on Acetaminophen induced hepatotoxicity in mice and this comparison was not found in previous studies.

The aim of present study was to compare hepatoprotective effects of *Cassia fistula* leaves and Silymarin on acetaminophen induced hepatotoxicity.

Materials and Methods

An experimental study was carried out in the department of Pharmacology and Therapeutics, Multidisciplinary research laboratory of Islamic International Medical College in collaboration with National Institute of Health Sciences (NIH), Islamabad from Mar 2015 to Mar 2016. Approval of synopsis was authorized by the Ethical Review Committee of Islamic International Medical College. Mice weighing 30-50grams with normal ALT and AST levels were included in the study and mice weighing less than 30grams and more than 50grams with abnormal liver function tests were excluded. Sixty Balb/c healthy albino mice were selected randomly by balloting method. A perfect living condition indistinguishable to their class with adjusted dietary supplement was given under temperature of 24±2ºC with a 12 hour light and dark cycle.

Cassia fistula leaves were collected from Race course park, Rawalpindi. The leaves were air dried and then authenticated by herbarium department of National Agricultural Centre, Islamabad. The external dirt of freshly collected leaves was removed by washing with tap water. The leaves were shade dried and crushed coarsely by hand. About 400 gm of these leaves were boiled in distilled water for 30 minutes. These were kept for 3 days with intermittent shaking. These were filtered by Whatman No1 filter paper. The aqueous extract was obtained by concentrated the filtrate by rotary flash evaporator.[17,19]

The mice were divided into four groups randomly, each group consisting of 15 mice. Mice in Control Group A were given normal diet and fresh water orally. Experimental Group B was given Acetaminophen 100mg/Kg orally in diet for 6 weeks once daily.[20] Experimental Group C was treated with acetaminophen 100mg/Kg with concurrent 400 mg/kg body weight of *Cassia fistula*(Aqueous Extract) orally for 6 weeks once daily.[21] Experimental Group D was treated with acetaminophen 100mg/Kg orally with concurrent 750 mg/kg BW of Silymarin in form of aqueous suspension in 2% Cremophor1E for 6 weeks once daily.[22] After six weeks cardiac puncture was done and blood samples were taken for evaluation of Serum Alanine Aminotransferase (ALT) and Serum Aspartate Aminotransferase (AST) levels.

The data was analyzed by using Statistical package for social sciences (SPSS) version 22. The biochemical parameters ALT and AST were calculated by using Mean ± Standard deviation (SD). The mean difference between control and other groups was calculated by applying ANOVA. The Post Hoc Tuckey test was applied for comparison of mean difference between groups. *p*-value 0f <0.05 was considered statistically significant.

Results

The serum ALT and AST levels were significantly raised (p<0.00) in Acetaminophen treated Group B as compared to control Group A. The serum ALT and AST were reduced in *Cassia fistula* treated Group C and Silymarin treated Group D and in comparison, the results showed significant reduction in serum

Table I: Comparison of Serum ALT Level between the Groups (n=60)

Serum Levels	Group A (control group)	Group B (positive control)	Group C (Cassia Fistula)	Group D (Silymarin)
ALT (Mean± SD)	37.30± 8.32	130.4± 42.90	62.22± 19.27	53.90± 25.06
AST (Mean± SD)	31.40± 10.05	134.3± 15.56	66.40± 20.50	44.67± 20.37

P value < 0.00 *P* value <0.05 =significant

Table II: Post hoc Tukey's test for multiple comparison of ALT between the Groups

Groups	Mean Difference	Significant
Group A vs Group B	-93.13	Yes
Group A vs Group C	-24.92	No
Group A vs Group D	-16.60	No
Group B vs Group C	68.21	Yes
Group B vs Group D	76.53	Yes
Group C vs Group D	8.322	No

Table III: Post hoc Tukey's Test for Multiple Comparison of AST between the Groups

Groups	Mean Difference	Significant
Group A vs Group B	-102.9	Yes
Group A vs Group C	-35.00	Yes
Group A vs Group D	-13.27	No
Group Bvs Group C	67.93	Yes
Group B vs Group D	89.67	Yes
Group C vs Group D	21.73	Yes

levels of ALT and AST in Silymarin treated Group D as compared to *Cassia fistula* treated Group C.

Discussion

Drug induced liver injury is an unresolved health problem and Acetaminophen is known to cause hepatic damage at high doses.[23] The reactive metabolite NAPQI has been implicated to play a role in hepatic damage.[8,24] The present study is designed to compare hepatoprotective effects of aqueous extract of *Cassia fistula* leaves and Silymarin on Acetaminophen induced hepatotoxicity by measuring biochemical parameters including serum ALT and AST. In present study hepatotoxicity is induced in Group B with Acetaminophen. Group C and Group D were given aqueous extract of *Cassia fistula* leaves and Silymarin with concurrent Acetaminophen respectively to ameliorate the hepatotoxic effects of Acetaminophen on serum levels of ALT and AST.

In the present study mice in experimental group B showed abnormal increase in serum levels of ALT and AST which were given Acetaminophen. This is in accordance with findings of Amir Mohammad Kazemifar et al., who induced hepatotoxicity in male Sprague-Dawley rats with single oral dose of Acetaminophen 800mg/kg by gavage with an orogastric canula and he observed significant increase in levels of ALT and AST.[25] This is also consistent with study of Abel Felipe Freitage who found significant changes in LFTs due to hepatotoxic effect of Acetaminophen.[26]

In present study significant improvement *($p < 0.05$)* in serum levels of ALT and AST in Group D is observed which was given Acetaminophen followed by Silymarin. This was also revealed by study of Amir Mohammad Kazemifar et al., who demonstrated the hepatoprotective property of Silymarin in Acetaminophen induced hepatotoxicity in male Sprague-Dawley rats.[25]

In present study a significant reduction ($p < 0.05$) was observed in raised serum levels of ALT and AST in Group C which was given Acetaminophen followed by *Cassia fistula* as compared to Group B which was given Acetaminophen. The hepatoprotective effect of ethanolic extract of *Cassia fistula* leaves was observed in study of Pradeep et al. at dose of 500mg/kg/day for 7 days against subacute Carbon Tetrachloride (CCl4) induced hepatic injury in rats. He found significant improvement in levels of ALT, AST.[17] Das et al. also proved the hepatoprotective effect of aqueous extract of fruit pulp of *Cassia fistula* against liver damage induced by CCl4 in Albino rats.[18] Our findings are in accordance with study of Jehangir et al. who observed significant reduction in biochemical parameters including ALT, AST, ALP and total bilirubin by ethanolic extract of *Cassia fistula* leaves against hepatotoxicity induced by Isoniazid and Rifampicin.[27] The better hepatoprotective effect of aqueous extract of *Cassia fistula* leaves could be proven with increasing duration of study. Further studies are required to see hepatoprotective effect of *Cassia fistula* leaves with higher doses and by use of different routes of administration. The molecular mechanism responsible for reducing biochemical and histopathological parameters can be explored by further studies.

Conclusion

In present study significant hepatoprotective effect was shown by aqueous extract of *Cassia fistula* leaves probably due to presence of flavonoids. Silymarin demonstrated better hepatoprotective effect than *Cassia fistula* against Acetaminophen induced hepatotoxicity.

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