RESEARCH ARTICLE

HEPATOPROTECTIVE EFFECT OF CAFFEINE AGAINST ISONIAZID-INDUCED HEPATIC DAMAGE IN ALBINO RATS

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ABSTRACT

Objective: The study was designed to investigate the hepatoprotective activity of caffeine against isoniazid-induced hepatotoxicity in rats.

Materials & Method: Hepatotoxicity was induced in albino rats by administering isoniazid (250 mg/kg, p.o.) once daily for 14 days. Simultaneously, caffeine (100, 200, 300 mg/kg p.o) was administered 1 h prior to the administration of isoniazid (250 mg/kg, p.o.) once daily for 14 days. Silymarin (50 mg/kg, p.o) was used as a reference drug.

Results: Elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin following isoniazid administration were significantly lowered due to pretreatment with caffeine.

Conclusion: The results of this study indicated that the hepatoprotective effect of caffeine might be attributed to its safe and effective treatment of isoniazid hepatotoxicity.

INTRODUCTION

The liver is a vital organ present in vertebrates and some other animals. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The direction of bile flow is opposite to the direction of the blood flow through the sinusoids [1]. This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, detoxification, and production of biochemical's which is necessary for body [2].

Anatomy of liver

The liver is the largest organ of the human body (Fig. 1), weights approximately 1500 g. It is located in the upper right corner of the abdomen. The organ is closely associated with the small intestine, processing the nutrient-enriched venous blood that leaves the digestive tract [3]. The liver is divided into 4 lobes: right, left, caudate and quadrate. The right and left lobes are the largest, while the caudate and quadrate are smaller and located posteriorly. Two ligaments are visible anteriorly. The falciform ligament present superiorly, which separates the right and left lobes. The round ligament sitituated inferior to the falciform ligament, which protrudes from the liver slightly. Adjacent to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the portal hepatic, where the hepatic artery and hepatic portal vein enter the liver. The portal vein carries nutrient laden blood from the digestive system. Inferior to the portal hepatic is the bile duct which leads back to the gallbladder [4].

Microscopic Anatomy of liver

The basic functional unit of the liver is the liver lobule (Fig. 2). A single lobule is hexagonal in shape and its size similar to the sesame seed. It is made up of liver cells arranged in one-cell-thick plate like layers that radiate from the central vein to the edge of the lobule [5]. The primary structures in a lobule include plates of hepatocytes (liver cell) form the bulk of the lobule, portal triads at each corner of hexagon, central vein, liver sinusoids that run from the central vein to the portal

triads, hepatic macrophages (Kupffer cells), bile canaliculi ("little canals")-formed between walls of adjacent hepatocytes, space of disse-a small space between the sinusoids and the hepatocytes [6].
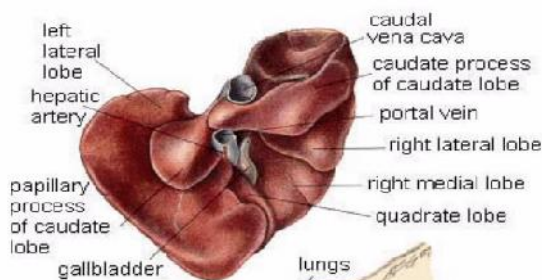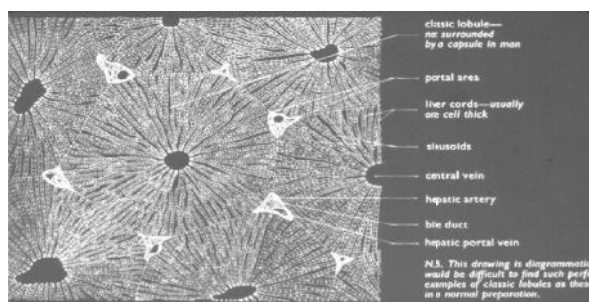

Figure 1 Anatomy of liver

Figure 2 Microscopic Anatomy of liver

Liver Cell Types

The liver has 5 cell types: Hepatocytes, kupffer cells, sinusoidal endothelial cells, bile duct epithelial cells, and ito cells. Most of the liver's synthetic and metabolic capabilities stem from the work of hepatocytes. Kupffer cells are macrophages that reside in the sinusoids. These cells help to clear out old red blood cells and bacteria. These cell help in breakdown of heme (the iron-containing pigment in haemoglobin) into bilirubin, which is one of the chief pigments of bile[1]. Sinusoidal endothelial cells have large pores that allow most proteins to pass freely through the sinusoidal endothelium into the space of Disse, where they can make direct contact with hepatocytes. Bile duct epithelial cells line the interlobular bile ducts within the portal triads. Ito cells are found in the space of disse. They are important because when the liver is injured, the Ito cells transform into cells that produce collagen, which leads to liver fibrosis. If this occurs on a large scale, it can lead to cirrhosis of the liver [1].

Liver disease

Liver disease is condition that causes liver inflammation or tissue damage and affects liver function. Liver disease is categorized both by the cause and the effect on the liver. Causes may include infection, injury, exposure to drugs or toxic compounds, an autoimmune process, or a genetic defect that leads to the deposition and build-up of damaging substances such as iron or copper. Effects may include inflammation, scarring, obstructions, clotting abnormalities, and liver failure. There are various liver disease e.g. hepatitis [7,8,9,10,11,12], acute liver failure [13,14], liver cancer [15,16], cirrhosis [17][18][19], budd-chiari syndrome[20], genetic [21,22], autoimmune liver disease [23]. Alcoholic liver disease [24, 25, 26] obstruction [27].

MATERIALS AND METHODS

Caffeine:

Caffeine was provided by truba institute of pharmacy bhopal

and purchased from K.M. traders, bhopal. The lethal dose of caffeine is too high to be of daily concern; the lethal dose is 170 mg/kg, or 12.5-14.6 g for an average adult male.

Isoniazide

Solonex is Isoniazide tablets. It was manufacture by macleod's pharmaceutical ltd and purchased from das medical store, karod (Bhopal). Hepatotoxicity was induced by daily dose of Isoniazide (250mg/kg p.o.) for 14 days.

Silymarin

Silymarin provided by Truba institute of pharmacy Bhopal. Silymarine manufactured by sigma aldrich and parches from K.M. treders bhopal (M.P.)

Animal care and handling

Westar albino rats of either sex weighing 150-250 g will use to observe for the hepatotoxicity and hepatoprotective activity of isoniazide and caffeine. The animals provide by animal house of Truba institute of Pharmacy, Bhopal. The animal were acclimatized to the standard laboratory condition in well cross ventilated animal house at temperature 25±2˚C relative humidity 44-56% and light and dark cycle of 10 and 14 hour respectively for one week before and during the experiment. The animal was fed with standard diet and water ad libitum. The experiment was approved by CPCSEA and the institutional ethics committee.

Grouping and dosing

Albino Westar rats of either sex (150-250g) will used. All the animals will divide into the six groups each group consists of 6 animals and they will receive the treatment as follows.

Group I: Control will receive propylene glycol for 14 days
Group II: Test will receive INH (250mg/kg p.o.) for 14 days
Group III: Standard will receive Standard drug (Silymarine 100 mg/kg, ip) + INH (250mg/kg p.o.) for 14 days
Group IV: Receive caffeine (100 mg/kg orally) + INH (250mg/kg p.o.) for 14.days
Group V: Receive caffeine (200 mg/kg orally) + INH (250mg/kg p.o.) for 14 days.
Group VI: Receive caffeine (300 mg/kg orally) + INH (250mg/kg p.o.) for 14 days.

Procedure for induce hepatotoxicity

250 mg/kg Isoniazide administer orally for 14 day. The test drug will administered orally by suspending in water solution. Twenty-four hours after last dose of INH, bloods will obtain from all groups of rats by puncturing retro-orbital plexus. The blood samples will allow coagulating for 45 min at room temperature. Serum will separate by centrifugation at 3000 rpm at room temperature for 20 min and used for the biochemical estimation given in fig. 3, 4, 5, 6.

Biochemical estimation

The blood samples were collected without any anticoagulant and were allowed to clot for 10 min. at room temperature. The blood was centrifuge at 3000 rpm for 10 min up to 4˚C. The

obtained serum was stored at 4°C for estimation of SGOT, SGPT and total bilirubin. This estimation was done according to the standard procedure given along with the perches kit (span diagnostics ltd.).
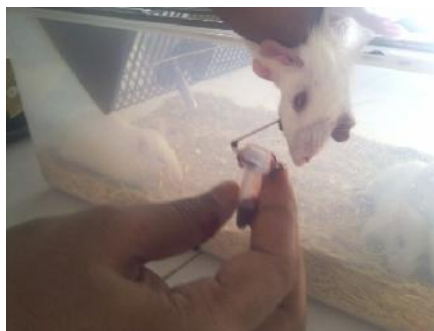

Figure 3 Mild anesthesia by chloroform

Figure 4 Collection of blood sample

Figure 5 Blood sample

Figure 6 Working with cooling centrifuge

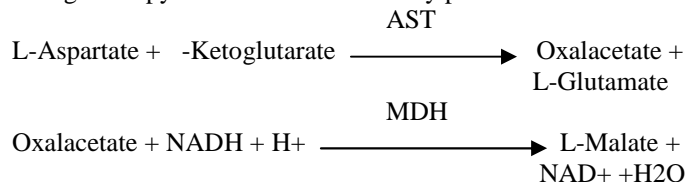The following serum enzymes will be measured:

Alanine aminotransferase (ALT/ SGPT)
Aspartate aminotransferase (AST/SGOT)
Total bilirubin (TB)

Estimation of serum SGOT

Principle

Aspartate aminotransferase (AST) catalyzes the transfer of the

amino group from L-aspartate to -Ketoglutarate to yield oxalacetate and L-glutamate. The oxalacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase (MDH) catalyzed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

$$L\text{-Aspartate} + \text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + L\text{-Glutamate}$$

$$\text{Oxalacetate} + NADH + H+ \xrightarrow{\text{MDH}} L\text{-Malate} + NAD+ + H_2O$$

Working reagent preparation:

Reagent 1, 2 and 4 are ready to use
Solution 1: dilute 1ml of reagent 3 to 10 ml with purified water.
Reagent storage and stability:
Reagent 1, 2 and 4 are stable at 2-8°C and reagent 3 is stable at 15-30°C till the expiry date mentioned.
Solution 1 is quite stable at room temperature (15-30°C)

Procedure

Estimation of serum SGOT given in table 1.

Formula

$$SGOT = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \text{concentration of standard}$$
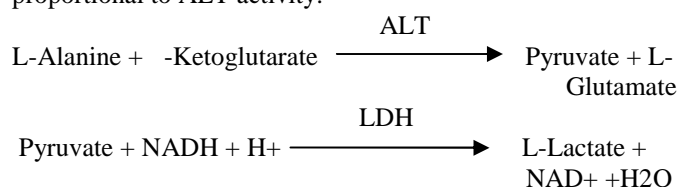
Table 1 Estimation of serum SGOT

Pipette in to the marked	Blank	Standard	Test	Control
		Volume in ml		
Reagent 1	0.25	0.25	0.25	0.25
Serum	---------	---------	0.05	----------
Standard	----------	0.05	----------	----------
Mix well and incubate at 37 °C for 60 minutes				
Reagents 2	0.25	0.25	0.25	0.25
Deionised water	0.05	----------	----------	----------
Serum	----------	----------	----------	0.05
Mix well and allow to stand at room temperature (15- 30°C) for 20 minutes				
Solution 1	0.25	0.25	0.25	0.25

Estimation of SGPT

Principle

ALT catalyzes the transfer of the amino group from L-alanine to - ketoglutarate resulting in the formation of pyruvate and L-glutamate. Lactate dehydrogenase catalyzes the reduction of pyruvate and the simultaneous oxidation of NADH to NAD. The resulting rate of decrease in absorbance is directly proportional to ALT activity.

$$L\text{-Alanine} + \text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + L\text{-Glutamate}$$

$$\text{Pyruvate} + NADH + H+ \xrightarrow{\text{LDH}} L\text{-Lactate} + NAD+ + H_2O$$

Working reagent preparation:

Reagent 1, 2 and 4 are ready to use
Solution 1: dilute1ml of reagent 3 to 10 ml with purified water.
Reagent storage and stability:
Reagent 1, 2 and 4 are stable at 2-8°C and reagent 3 is stable at 15-30°C till the expiry date mentioned. Solution 1 is quite stable at room temperature (15-30°C)

Procedure

Estimation of SGPT given in table 2.

Formula:

$$SGPT = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \text{Concentration of standard}$$

Table 2 Estimation of SGPT

Pipette in to the marked	Blank	Standard	Test	Control
	Volume in ml			
Reagent 1	0.25	0.25	0.25	0.25
Serum	---------	---------	0.05	----------
Standard	----------	0.05	----------	----------
Mix well and incubate at 37 °C for 30 minutes				
Reagents 2	0.25	0.25	0.25	0.25
Deionised water	0.05	----------	----------	----------
Serum	----------	----------	----------	0.05
Mix well and allow to stand at room temperature (15- 30°C) for 20 minutes				
Solution 1	0.25	0.25	0.25	0.25

Estimation of serum Bilirubin:

Principle

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink colored azobilirubin with absorbance directly proportional to Bilirubin concentration.

Reagents:

Reagents 1: Diazo-A
Reagents 2: Diazo-B
Reagents 3: Diazo Blank
Reagents 4: Methanol
Reagents 5: Artificial Slandered (10 mg % Bilirubin)

Preparation of working solution:

Diazo Reagents: just before use mix 1.0 ml of reagent 1with 0.03 ml of reagent 2

Procedure

Estimation of serum Bilirubin given in table 3.

Read optical density agents distilled water at 540 nm wavelength.

Formula

$$\text{Total A} = \frac{\text{Optical density of T1} - \text{Optical density of T2}}{\text{Optical density of standard}} \times 10$$

Table 3 Estimation of serum Bilirubin

Reagents	T1	T2
Serum/plasma, ml	0.05	0.05
Distilled Water, ml	0.45	0.45
Reagents3: Diazo Blank, ml	--	0.125
Diazo Reagents, ml	0.125	--
Distilled Water, ml	--	--
Reagent 4: Methanol, ml	0.625	0.625

Note: Read optical density agents distilled water at 540 nm wavelength.

RESULTS AND DISCUSSION

The prime objective of study was to investigate the hepatoprotective activity and effective dose of caffeine used to reduce the liver injury. The hepatoprotective activity screened by carbon tetrachloride, paracetamol and Isoniazide induced hepatotoxicity in male wistar albino rat. In this protocol hepatoxicity induce by oral administratation of Isoniazide in rat which leads to the hepatocellular necrosis in 14 day. The degree of liver damage was determined by biochemical parameter such as SGOT, SGPT, and total serum bilirubine and probably by histopathology. During metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetyl hydrazine).

It is evident that hydrazine plays a role in INH-induced liver damage. It can cause moderate abnormalities in serum transaminase leading to hepatotoxicity; hence the measurement of serum transaminase is often advocated during INH administration, to assess the extent of INH-induced hepatotoxicity [28]. Administration of INH significantly elevated levels of AST, ALT, bilirubin, due to damaged structural integrity of the liver because these are cytoplasm in location and are released into circulation after cellular damage [29].

Caffeine treatment with INH prevented the INH-induced perturbations in the activities of AST, ALT, TB, in both the serum and liver tissue. It is likely that the INH-derived hepatotoxicity metabolites, namely acetyl. The results in this study were confirmed by Histopathological observation. In contrast to the control group, INH-intoxicant rat showed mild inflammatory cell infiltration and fatty changes, but rimonabant administration for 14 days attenuated this histopathological changes. Administration of INH at a dose of 250 mg/kg (p.o.) caused a significant rise (P<0.01) in level of serum marker enzymes such as AST, ALT, TB, Silymarin significantly (P<0.01) reduced these levels near to normal. A significant (P<0.01) decrease was observed in the AST, ALT, TB (Table 1) level in the animals treated with different doses of caffeine (10 mg/kg, 20 mg/kg and 30 mg/kg) and showed dose dependant activity. At the dose of 20 mg/kg of caffeine showed comparable activity with the standard drug silymarin.

The observed Histopathological changes under medical microscope at 100X and then photomicrography of liver of experimental groups are shown in fig. 7,8,9,10,11,12. Group 1 showed the histopathology of normal control rats which showed in control rat, liver section showed normal hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus and central vein. The INZ group II treated rat liver showed there was hydropic change hepatocyte with single cell necrosis surrounded by neutrophils. Congestion of central vein and sinusoid were seen with acute inflammatory cell in filtering sinusoids mainly in central zone. The INZ +

Table 4: Effect of caffeine on serum biochemical parameter in Isoniazide induced hepatotoxicity in rat

Group	Treatment	Dose	SGOT(U/L) mean ± SEM	SGPT(U/L) mean ± SEM	Total bilirubine (mg/dl) mean ± SEM
I	Vehicle	0.2 ml of propylene glycol	60.30 ± 1.22	46.06 ± 1.49	30.95 ± 1.68
II	INZ	250 mg/kg	152.50 ± 4.41 a***	92.83 ± 8.68 a***	52.94 ± 1.38 a***
III	Silymarin+ INZ	100 mg/kg + 250 mg/kg	65.57 ± 3.34 b***	49.59 ± 1.41 b***	34.21 ±1.46 b***
IV	Caffeine+ INZ	10 mg/kg + 250 mg/kg	127.58 ± 6.61 a***c***	77.07 ± 2.67 a**c**	48.23 ± 2.46 a***c***
V	Caffeine+ INZ	20 mg/kg + 250 mg/kg	85.66 ± 1.94 b***d**	51.67 ± 7.39 b***d*	40.70 ± 1.76 a**b***
VI	Caffeine+ INZ	30 mg/kg + 250 mg/kg	116.90 ± 16.21 a***b*c***	71.63 ± 4.21 a*	45.82 ± 1.55 a***c***

Silymarine treated (group III) rat liver there was milled central venous congestion and milled fatty vacoulation.

Caffeine of dose 20 mg/kg show significant result as compared to other dose of caffeine treatment for 14 day as there liver section showed residual hepatocellular necrosis with cords of regenerating hepatocytes. INZ induce hepatotoxicity showing extensive area of confluent necrosis and also showing fatty change and hydropic degeneration.

Pre-treatment with silymarine showing complete normalization of liver architecture. Pre-treatment with caffeine showing better histology for 14 day treatment as there liver section showed partial protection of hepatocytes. Effect of caffeine on serum biochemical parameter in isoniazide induced hepatotoxicity in rat given in table 4. The observed Histopathological changes under medical microscope at 100X and then photomicrography of liver of experimental groups are shown in fig. 7,8,9,10,11,12 and result shown in graph 1.

No of animal in each group (n) = 6 INZ = Isoniazide

The data were analyzed by one way ANOVA followed by tukey multiple comparison Test.

Each values represents the mean ± SEM; n=6, *p<0.05, **p<0.01, ***p<0.001

- a- Significant difference as compare to normal control group
- b- Significant difference as compare to negative control group
- c- Significant difference as compare to standard group
- d- Significant difference as compare to test I group
- e- Significant difference as compare to test II group
- f- Significant difference as compare to test III group
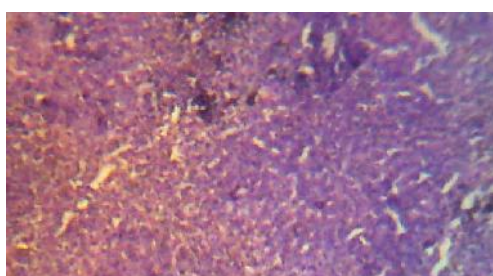
Histopathological study
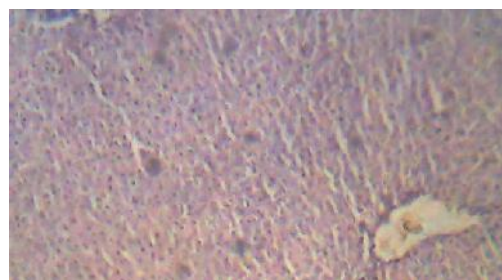

Figure 7 Normal group

Figure 8 INZ treated group
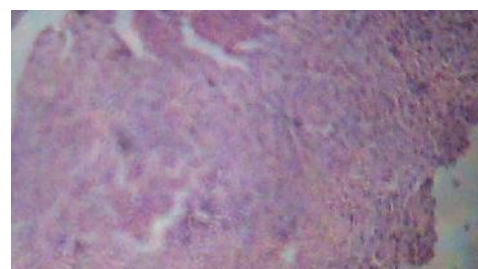

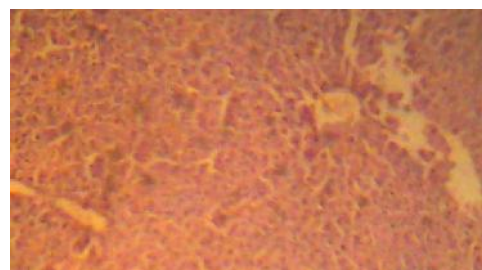**Figure 9** Silymarine + INZ treated group

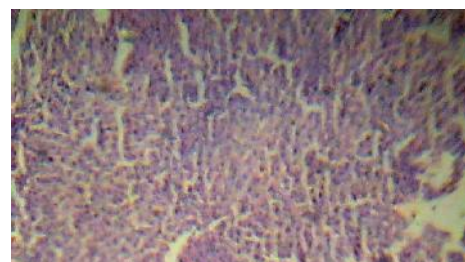**Figure 10** INZ + Caffeine (10 mg/kg) treated group

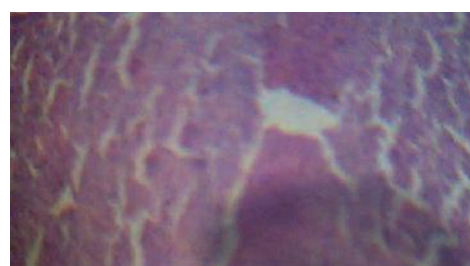**Figure 11** INZ + Caffeine (20 mg/kg) treated group

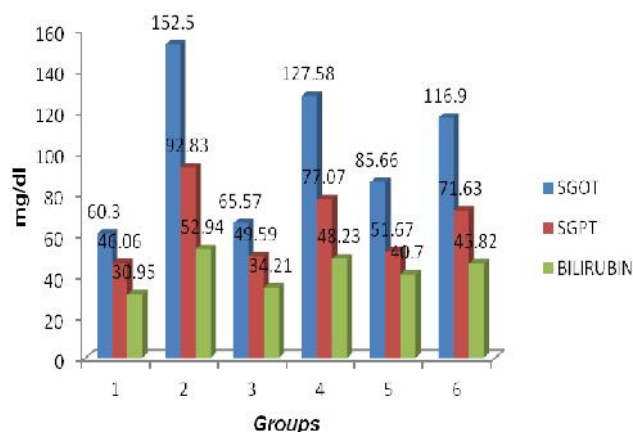**Figure 12** INZ + Caffeine (30 mg/kg) treated group

Figure 13 Effect of silymarine and caffeine on SGOT, SGPT, bilirubine level.

CONCLUSION

The study was designed to evaluate the hepatoprotective activity of caffeine and effective dose, against albino rat in experimental liver damage induced by Isoniazide. Caffeine show significant protective effect by lowering the serum level of SGOT, SGPT, and total bilirubine of the liver. This biochemical observation was supplemented by histopathological examination of liver section. The activity of various doses was comparable to that of silymarine, a known hepatoprotective. In present pharmacological evaluation, the caffeine was extensively indigested for its hepatoprotective potential against INZ induces hepatotoxicity. At the end of this study, a strong conclusion can be drowning that caffeine posse's significant hepatoprotective activity. Caffeine of dose 20 mg/kg show better result as compared to 10 mg/kg and 30 mg/kg.

ACKNOWLEDGMENT

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