HEPATO-PREVENTATIVE EFFECTS OF *HIBISCUS SABDARIFFA* L. AGAINST HEPATO-TOXICITY INDUCED BY PARACETAMOL IN RATS

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ABSTRACT

The present work was carried out to unravel the hepato-preventative potential of *Hibiscus sabdariffa* L. against hepato-toxicity generated by paracetamol in experimental Wistar rats. Paracetamol has been used extensively to study hepatotoxicity in animals by initiating lipid peroxidation causing injuries to liver tissues. Holding a wide variety of biological activities, *Hibiscus sabdariffa* L. extract have shown antioxidant, antibacterial, chemo preventive, antipyretic, anti-inflammatory, anti-nociceptive, antifungal, anti-parasitic and laxatives effects. Healthy male Albino Wistar rats weighing 170-220 g were purchased from the animal house ICCBS for the study. Based upon the age and weight, 3 experimental groups were constructed (n=5). Group A served as untreated control whereas Group B and C received Paracetamol 0.9 mL/kg of body weight via gavage for 15 days. In addition Group C received Hibiscus extract orally on daily basis for 15 days. The experimental phase ended at 15th day. At the end of 15th day animals of group A, B and C were sacrificed and blood samples were used to assay concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate amino-transferase (AST), and bilirubin. Furthermore, part of the hepatic tissue was processed for histological investigation including fixation, paraffin embedding, and hematoxylin-Eosin staining. The fifteen days treatment of Albino rats with paracetamol (0.9 mL/kg) and *Hibiscus* extract (22gram percent/kg of body weight via gavage) have shown to significantly reduce the enzymes ALT, ALP, AST and bilirubin in Group C as compared to group B. Histopathological characteristics of the specimens suggested a protective effect of *Hibiscus sabdariffa* L. with amelioration of portal and peri portal fibrosis in the herb-treated group C. *Hibiscus sabdariffa* L. extract has been shown to protect liver fibrosis and cirrhosis induced by paracetamol. It is, therefore, concluded that daily usage of Hibiscus not only can improve health in normal individuals but also benefit healing process in patients with liver damage.

Keywords: *Hibiscus sabdariffa* L., Hepatopreventative, paracetamol, Histopathology.

INTRODUCTION

One of the well-known flower plants grown globally is *Hibiscus*. In the world 300 or more *Hibiscus* species are found and roselle (*Hibiscus sabdariffa* L.) is one of that specie of *Hibiscus*, which is a member of the plant family Malvaceae (Ismail *et al*., 2008). Possessing marked antioxidant potential, *Hibiscus* not only caused hepatic stellate cells' inhibition, but also considerably reduced the hepatic damage such as, fibrosis and steatosis in a dose dependent way. Proto-catechuic acid (a phenol found in the calyces of *H.sabdariffa*) owing to its oxidant scavenging characteristics and occluding the oxidant-signal transduction, has shown to have preventive effect against liver deterioration produced by tert-butyl-hydroperoxide (Liu *et al*; 2002). The Proto-catechuic acid (Tseng *et al*; 1996) and anthocyanins (Wang *et al*., 2000; Tsai *et al*., 2002) produced a protecting effect induced by tert-butyl-hydro peroxide in a culture of rat hepatic cells. Lipopolysaccharide (LPS) generated rat hepatotoxicity (Lin *et al*; 2003) and tert-butyl-hydro peroxide-induced rat hepatic damage (Liu *et al*; 2002) also inhibited by *Hibiscus* Proto catechuic acid. The *Hibiscus* has a lengthy history of uses throughout the world like in preventing renal stone formation, in the treatment of cancers (Tseng *et al*; 1996), respiratory diseases and, in the formation of warm and cold teas, jellies, drinks and jams (Da-Costa-Rocha *et al*., 2014; Okoro, 2007, Bolade *et al*., 2009) also for the treatment of nerve and heart diseases and has been used as treatment for constipation also used as diuretic (Osuntogun and Aboaba, 2004). It was observed in Iran to drink a sour tea as therapy for hypertension (FarajiandTarkhani, 1999).

Paracetamol over-ingestion is one of the leading causative factors in acute hepatotoxicity (Budnitz *et al*., 2011). When taken in therapeutically-acceptable dose, a pathway is activated causing paracetamol to be metabolized and advancing towards the virulent-metabolite formation, the N-acetyl-p-benzoquinone imine (NAPQI), which under these circumstances is detoxified by glutathione, preventing the cells from the deteriorating effects (Jaeschke *et al*., 2012); however, overdose of paracetamol leads to augmented NAPQI production which curtails hepatic GSH concentrations, adducts the proteins together which include the mitochondrial-associated proteins and give rise to

mitochondrial-dysfunction. A consequent DNA-splitting up occurs that eventually lead towards the immuno-cellular activation and release of cytokines (pro-inflammatory) and contributing in hepatotoxicity (Jaeschke *et al*., 2012).

Viruses cause liver damages such as hepatitis A, hepatitis B, hepatitis C and other hepatic damages can be due to the poisons or drugs (Navarro and Senior, 2006).In childhood stage 20-30% of hepatitis patients attained their infection. Paracetamol has been widely used to experimentally induce liver injury in rodents. After oral administration of paracetamol, it is then absorbed with-in one hour and reaches to peak level of plasma concentration. If taken in rapidly absorbed form or liquid it will take 30 minutes. Inactivation took place in hepatocytes by association leading to sulfate or glucuronide and excretion occurred through urine (Navarro and Senior, 2006).

Plants have a lengthy and rich history of medicinal uses and, even in the period of current medicine, medicinal properties of herbs are still required afterand have been used traditionally in different localities. Therefore, the focus of the present investigation was to evaluate the prospective hepatoprotective effects of *Hibiscus* in experimental rat models of liver hepatotoxicity induced by paracetamol.

MATERIALS AND METHODS

Male rats (Albino Wistar), weighing 170-220 gwere brought from the ICCBS [International Center for Chemical and Biological Sciences, Karachi, Pakistan]. Animals were become accustomed tothe laboratory condition prior to the initiation of the protocol. Accommodation of the animals was done in polycarbonated-cages with easy approach to water and diet.

MATERIALS AND EXTRACTION PROCEDURE:

Hibiscus flowers were collected from the different areas of Karachi and separated their petals. These petals were then air dried indoors under subdued light and with good ventilation and after 1 week they were crushed into a course powder using mixer. The powder was then boiled in distilled water to make 22.0g%.

ETHICAL GUIDELINES

Institutional Ethical Review Board's ethical guidelines were followed for the experiments with internationally acknowledged ethical practices in animal care in research (Health Research Extension Act of 1985).

STUDY DESIGN

Based upon the age and weight, 3 experimental groups were constructed (n=6). Group A served as control whereas Group B and C received Paracetamol 0.9 mL/kg of body weight, injected beneath the skin, for 15 days; whereas a daily dose of *Hibiscus sabdariffa* (250 mg/kg body weight) extract was given to Group C till 15 days. The experimental phase ended at 15^{th} day. At the end of 15^{th} day animals of group A, B and C were sacrificed; cardiac puncture was done with heparinized syringes for obtaining blood samples, which then were centrifuged. Blood samples were used to assay concentrations of Alanine aminotransferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Tietz *et al*., 1983), aspartate amino-transferase (AST) (Reitman and Frankel, 1957), and bilirubin levels (Dangerfield *et al*., 1953).

ASSESSMENT OF AST, ALT, ALP and BILIRUBIN

Plasma ALP, ALT, AST and bilirubin were evaluated by using reagent kits prepared commercially from Randox.

HISTO-PATHOLOGICAL INVESTIGATION

Part of the hepatic tissue was processed for histological investigation including formalin fixation, paraffin embedding, and hematoxylin-Eosin staining. Histological sections were then examined for the degree of hepatic injury according to French by the use of grading and numerical scores.

STATISTICAL ANALYSIS

Research outcomes were presented as mean± standard deviation. Significance of the difference between groups was obtained using independent sample T test.

RESULTS

Impact of paracetamol and *Hibiscus Sabdariffa* L. treatment on liver and body weight in the assigned groups of rats:

Major feature of injury is the reduction of weight of the body. Table1 compares the effects of Hibiscus among control, paracetamol treated and paracetamol + Hibiscus treated rats and illustrates that animals with paracetamol administration considerably reduced body weight in comparison with the control ($P<0.05$). Hibiscus treatment exhibited that body-weight is increased considerably in Hibiscus + paracetamol treated group as compared with paracetamol treated group ($P<0.05$). The liver weight ($p<0.05$) and the relative liver weight ($p<0.01$) had noticeably arisen in the paracetamol treated group in contrast with the control, whereas a significant curtailment in the liver weight ($P<0.05$) and the relative liver weight ($P<0.05$) was noticed in paracetamol + *Hibiscus* treated rats as compared with paracetamol.

Table 1. Comparison of body, liver and relative-liver weight in Control, Paracetamol-treated + *Hibiscus Sabdariffa* L.-treated rats.

Variable	Control	Paracetamol	Para+Hibiscus
Initial Body Weight	176.6 ± 6.179	181.3 ± 2.33*	193 ± 4.36*
Final Body Weight	165 ± 6.11	167 ± 1*	181.3 ± 3.93*
Liver Weight	3.96 ± 0.13	4.1 ± 0.15	4.0 ± 0.45
Relative Liver Weight	2.40 ± 0.14	2.45 ± 0.1	2.19 ± 0.23

*p-value < 0.05 as compared with control; n=6; Data presented as mean ± S.D.

Table 2. Comparison of Serum marker and Bilirubin for liver injury in Control, Paracetamol treated and Paracetamol + *Hibiscus Sabdariffa* L. treated rats.

Variable	Control	Paracetamol	Para+Hibiscus
AST	9.55 ± 1.81	16.03 ± 2*	11.4 ± 2.34*
ALT	11.42 ± 0.7967	14 ± 0.46*	12.4 ± 0.611*
ALP	95.22 ± 53.44	165.6 ± 16.78	141.63 ± 25.93*
Bilirubin	0.68 ± 0.311	2.01 ± 0.26*	0.75 ± 0.2*

*p-value < 0.05 as compared with control; n=6; Data presented as mean ± S.D.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase, AST, aspartate amino-transferase

Table 3. Histological features in Control, Paracetamol and Paracetamol + *Hibiscus Sabdariffa* L. treated rats.

Histo-pathological findings	Control	Paracetamol	Para+*Hibiscus*
Enlargement	1	1	1
Paleness	1	1	1
Fatty change	0	0	0
Hydropic degeneration	0	0	0
Periportal fibrosis	0	1	0
Bile Duct Proliferation	0	0	0
Dysplasia	0	0	0
Portal Fibrosis	0	1	0
Total	2	4	2

Extent of hepatic lesion is indicated as scores noticed using light microscopy. Score 0, no visible damage; Score 1, focal hepatic cell devastation on less than 25% of the tissue; Score 2, focal hepatic cell devastation on 25-50% of the tissue;Score 3, considerable focal hepatic cell devastation; Score 4, global hepatic cell necrosis.

Effects of Paracetamol and *Hibiscus* Treatment on Liver Enzymes (ALP, AST, ALT) and Bilirubin in the Assigned Groups of Rats:

Table 2 shows that hepatic enzymes ALP ($P<0.05$), AST ($P<0.05$), and ALT ($P<0.05$) levels were increased drastically in paracetamol treated rats as compared with control group. *Hibiscus* treatment has significantly reduced the enzymes ALT ($P<0.05$) and ALP ($P<0.05$) and AST ($P<0.05$) in Paracetamol + *Hibiscus* treated group as compared with paracetamol treated group. Table2 shows that bilirubin level in rats has significantly risen by paracetamol treatment as compared with control ($P<0.05$). Concentration of bilirubin was decreased significantly ($P<0.05$) in Paracetamol + *Hibiscus* treated group as compared with paracetamol treated group.

Histo-pathological Findings:

Changes in liver morphology was depicted, investigated and compared in the Control, Paracetamol-treated, and Paracetamol +*Hibiscus* treated groups. Paracetamol treated rat liver tissues shows severe histo-pathological changes. The most important changes include portal and periportal fibrosis. Hepatocytes degeneration was also noted and it constitute for 30-40% (Table 3; Fig 2). However enlargement and paleness were present in the hepatic lobules of control, paracetamol treated and paracetamol +*Hibiscus* treated groups (Table 3; Fig 1-3).

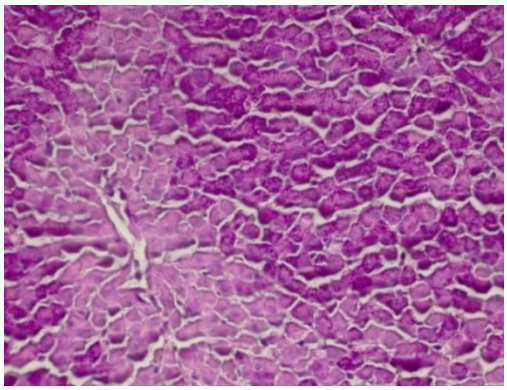

Fig. 1.Histo-pathological features of Control group of rats.

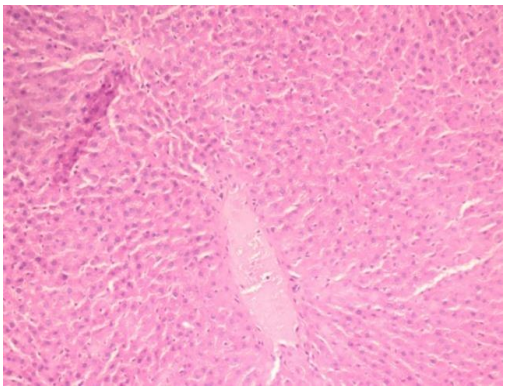

Fig. 2. Histo-pathological features of Paracetamol + *Hibiscus t*reated rats.

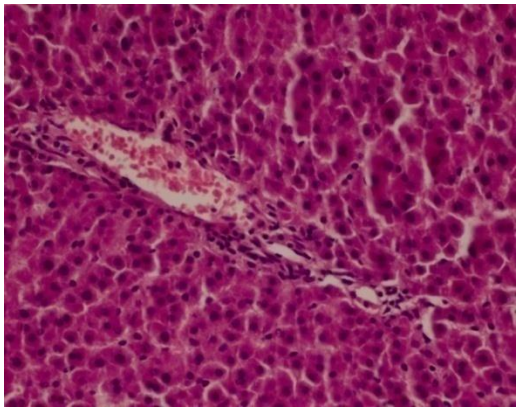

Fig. 3.Histo-pathological feature of Paracetamol treated rats.

DISCUSSION

The present study was carried out for investigating the protective potential of the selected herb i.e. *Hibiscus Sabdariffa* L. against Paracetamol-induced liver-toxicity in rats. The enzyme CYP2E1 when metabolizes paracetamol, leads to activation of this toxicologically significant substrate into a greater toxic product (French *et al*., 1997; Guengerich *et al*., 1991).Liver damage is not only due to the activation of enzymes in the liver by the paracetamol-administration and generation of toxic metabolites through cytochrome p450 system, but also due to the ingestion of drug itself. The virulent-metabolite, NAPQI is usually caused to become harmless through an interaction with the glutathione, an endogenous antioxidant. However, storage of glutathione in the liver becomes reduced when production of paracetamol metabolite is increased and the metabolite begins to accumulate and leads to liver injury (Dahlin *et al*., 1984; Jaeschke *et al*., 2003; Klopčič *et al*., 2015).

A medicinal herb and local soft drink material, extracts of desiccated flower *Hibiscus sabdariffa* L., were used to study their hepatoprotective effects against fibrotic liver induced by paracetamol in rats. Liver injury such as fibrosis and steatosis is reduced by the use of *Hibiscus*in a dose dependent way. Moreover, *Hibiscus* considerably reduced the plasma alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) ($p<0.05$). The anthocyanins of *H. sabdariffa* were also shown to have a protecting effect against tert–butyl hydro peroxide-induced hepatic toxicity in rats(Wang *et al*., 2000; Tsai *et al*., 2002). The anthocyanins were able to quench the free radicals of 1, 1-diphenyl-2-picrylhydrazy. Moreover there are many reports of its biological activities including:antipyretic, anti-nociceptive and anti inflammatory activities (Reanmongkol and Itharat, 2007), antioxidant (Tsai *et al*., 2002), anticancer activity (Tseng *et al*; 1996),and antifungal, anti-parasiticand antibacterial effects (Liu *et al*., 2005).

In our study, when we compared the weights of these three groups then it was observed that weight of paracetamol treated animals was highly reduced as compare to *Hibiscus* + paracetamol treated animals ($p<0.05$)and least reduced weights were observed in control group ($p<0.05$). In the study when the effects on liver enzymes were observed then it has been shown that after treatment with paracetamol, liver enzymes ALT, AST, ALP and bilirubin were considerably increased ($p<0.05$).Liver metabolic enzymes' elevation indicates liver dysfunction due to the damage of cell and enzymes escape into the blood. On the other hand cell damage and its function is due to the loss of lysosomal and mitochondrial membranes of the liver and increased levels of serum bilirubin indicate the serious liver injury.

Administration of *Hibiscus* with paracetamol has been revealed to reduce ALT, AST, ALP and bilirubin concentration thereby suggesting its protective effects against hepatic damage thus, *Hibiscus* prevents damage of rat liver tissue structure. Moreover, this study showed that the histological findings are in accordance with the biochemical results. Histological changes in liver tissues confirmed the liver injury induced by paracetamol and showed portal fibrosis with degeneration of hepatic cells and intracellular pigment deposition. Our study showed that treatment of *Hibiscus* along with paracetamol has, in part, improved, and paracetamol induced histological changes of the liver has reversed (Table 3; Fig 1-3).

As in our population incidences of infections specially hepatitis have been increased day by day due to poor hygiene and illegally used contaminated devices. It is, therefore, concluded that *Hibiscus* has shown to exert protection against liver damage induced by fibrosis and cirrhosis of liver and its daily usage maybeneficial in healing process in patients with liver damage.

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