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Protective effect of *Heliotropium strigosum* 70% aqueous methanolic extract against paracetamol induced hepatotoxicity in mice

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Abstract: The study was carried out to evaluate the hepatoprotective potential of aqueous methanolic extract of *Heliotropium strigosum* (*HSME*) against paracetamol induced hepatotoxicity in Swiss albino mice. The plant powder (1.5Kg) was macerated in aqueous methanol (30:70) for 7 days. The extract was evaluated for the presence of different phytochemicals and High-performance liquid chromatography (HPLC) analysis. *HSME* was orally administered to mice at 125, 250 and 500mg/kg for 8 days followed by paracetamol intoxication (500mg/kg orally) on the 8th day using silymarin as standard control. All the therapy was administered by oral gavage. The liver biochemical parameters and histopathological evaluation were carried out to assess changes in liver function and histology. HPLC analysis confirmed the presence of quercetin, kaempferol, and other phenolic compounds. Treatment with the extract resulted in notable ($p<0.05$) reduction in liver parameters in dose dependent manner. The action of HSME 500mg/kg dose was comparable to silymarin. The effect of *HSME* against paracetamol induced hepatotoxicity was demonstrated by protective changes in the liver histopathological which proved the traditional uses of the plant.

Keywords: Hepato-protection, HPLC, kaempferol, *Heliotropium strigosum,* histopathology.

INTRODUCTION

The liver has a crucial function as a blood purifier. Several pathogens such as viruses, parasites as well as, herbs, xenobiotic or medicines when taken in high doses damage the liver due to its task as a first line defence in the body (Saleem *et al.* 2018). More than 900 toxins, drugs and herbs were reported to be hepatotoxic. The primary harmful agents include heavy metals, antibiotics, anticonvulsants and antipsychotics (Björnsson 2015). Some drugs such as colchicine, interferon, corticosteroids, silymarin, curcumin, Resveratrol and Liv-52 are effective in the management of hepatic dysfunction, viral hepatitis or drug induced hepatic diseases (Saleh *et al.,* 2017).

Many people prefer herbal medicines over synthetic medicines due to ethnical beliefs, low cost and less severe side effects (Fatima *et al.,* 2019). Preference for herbal drugs is increasing for several decades. The usage of medicinal plants is common in Asia and Africa. According to the report by World Health Organization, people are increasingly relying on natural products in developed countries because of the adverse effects accompanying with allopathy (Janbaz *et al.,* 2015). However, a lack of pharmacological validation and toxicological evaluation of a huge number of medicinal plants, places the consumers under unnecessary risk of irrational drug use and intoxication (Janbaz *et al.,* 2015).

Heliotropium strigosum Willd belongs to Borginaceae family. The genus *Heliotropium* comprises of 270-275 species growing in tropical and temperate regions. Twenty-three species of this genus grow in Pakistan (khuram *et al*., 2016). The plant *H. strigosum is commonly known as Gorakh Pam, Kharsan and Bhangra in urdu in Pakistan. H. strigosum* is used as herbal medicine in West Africa for treating abscesses of breasts (Ghori *et al*., 2016). It was reported to contains an alkaloid strigosine (Saeed *et al.* 2017). Some literatures also reported the used of this plant in the management of body pain, gynaecological complications, renal disorders, achy eyes, nettle stings, gum abscess, traumatic wound as well as, snake and insect bites (Gouda and Abdelazeem 2016). The whole plant was reported to be conventionally used in the treatemts of jaundice and rheumatoid arthritis as well as blood purifier, diuretic and laxative (Khuram *et al*., 2016). Previous studies reported the use of *H. strigosum* in the treatment of *respiratory,* cardiovascular and gastrointestinal ailments. Therapeutic effects such as antipyretic, anti-inflammatory, anti-diabetic, anti-

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dyslipidaemia, antimicrobial, antioxidant, cytotoxic, phytotoxic, antiglycation and insecticidal activities effect of this was also reported (Basak and Dey 2016).

Literature survey showed that no prior study authenticating the hepto-protective potential of *H. strigosum.* Furthermore, the plant has shown *in vitro* antioxidant activities in previous investigations (Shah *et al.,* 2014). Therefore, the aim of this study was to evaluate the hepatoprotective potential of this plant in mice and also study the bioactive constituents present in this plant through High performance liquid chromatography (HPLC).

MATERIALS AND METHODS

All the chemicals used in the study were of analytical grade. Solvents used in HPLC analysis were of HPLC grade. Shimadzu HPLC Japan was used in the study.

Preparation* of *HSME

Fresh plant *H. strigosum* (4kg) was collected from Faisalabad in July, 2016. The plant specimen was identified (Voucher number 259-1-2017) and deposited in the herbarium of University of Agriculture, Faisalabad for further reference. The plant powder (1.5kg) was macerated in aqueous methanol (30:70) for 7 days with continuous shaking daily. Filtration was done using muslin cloth then filtrate was filtered through Whatsman filter paper grade 1 (11µm). The filtrate was evaporated with a rotary evaporator (Cole-Parmer®, UK) at 40 °Cunder reduced pressure. The extract (11.3% yields) was labelled and preserved in an air tight container at -8°C until further use (Saleem *et al*., 2020).

% yield = Weight of dry extract*100/weight of dry plant taken

Qualitative phytochemical evaluation

The qualitative phytochemical evaluation was carried out on the *H. strigosum* aqueous methanolic extract *(HSME*). The detection tests were performed for reducing sugars, alkaloids, terpenoids, tannins, flavonoids, saponins, steroids, proteins, carbohydrates and glycosides according to methods described previously (Fatima *et al.,* 2019).

HPLC analysis

Quantitative phytochemical analysis was performed to determine the quantities of flavonoid and phenolic compounds present in the extract using HPLC (Shimadzou®, Japan) according to previous method (Saleem *et al.* 2020). The *HSME* (50 mg) was dissolved in 5 ml distilled water (DW). Methanol (12 ml) was added to the extract solution and shaken properly. The mixture was allowed to stand for 5 min followed by the addition of DW (6 ml). The solution was further kept for 5 min at 37°C. Then 10 ml of 15 M hydrochloric acid was added drop wise. The acidified extract solution was incubated again for 2h at 90°C. The solution was filtered with 0.2 µm syringe filter. Acetonitrile, dichloromethane and methanol (60:20:20) constituted the mobile phase used in HPLC analysis. The flow rate was maintained at 1 ml/min during the process. Phenolic and flavonoids were detected by HPLC using a C18 column at 280 nm with a UV-Visible detector. Phytochemicals were detected and quantified in comparison to the retention time of the relevant reference compound.

In vivo experiment

Adult Swiss albino mice of both sexes, weighing 25-30 g, were acquired from University of Agriculture, Faisalabad. The mice were housed in the Animal house of Government College University Faisalabad. Mice were given a standard pellet chow diet and water ad libitum. Standard temperature 25±2°C and 45-55% humidity were maintained throughout the experiment. Mice were allowed to acclimatized in laboratory conditions for two weeks. The study was approved by the Institutional Ethics Committee of GC University Faisalabad (Ref. No. GCUF-ERC-1678).

Acute toxicity study

Five groups of mice each comprising five animals were used to assess the acute toxicity of *H. strigosum* extract. Group 1 assigned as control group was given DW only. The four groups were administered with 500, 1000, 1500 and 2000 mg/kg extract in single oral dose. The mice were observed in for lethality, adverse effects and behavioural changes for 72 h (Saleem *et al*., 2018).

Hepatoprotective potential

The *HSME* was dissolved in distilled water (DW) for use in mice. Doses were reconstituted according to the average mouse body weight and administered with oral gavage.

For 125mg/kg dose=18.75mg of extract /2.5ml, for 250mg/kg = 37.5mg of extract/2.5ml and for 500 mg/kg= 75mg of extract/2.5ml were dissolved in DW. Paracetamol (1g/Kg) suspension was prepared with 0.5% w/v gum tragacanth in normal saline to increase solubility (Saleem *et al*., 2018).

Experimental procedure

Swiss mice were randomly divided into six groups. Each group contained 5 mice. Group one was treated as a normal control group. The DW 0.5ml/kg was administered to group one and two per os daily for eight days. Group 2 served as a paracetamol control group. Group 3 was treated with silymarin (standard reference) (100mg/kg) for 8 days. Animals of group 4, 5 and 6 received 125, 250 and 500mg/kg *HSME* respectively for eight days by oral gavage. Paracetamol (500mg/kg) was given orally to all mice 3 h after administration of daily therapy on the 8th day with the exception of group one (control) (Saleem *et al*., 2018).

Assessment of biochemical parameters

Mice were anesthetized by inhalation with chloroform dipped cotton swab placed in a closed container 48 h after paracetamol administration. Blood (1.5ml from each mouse) was collected by cardiac puncture for estimation of liver function tests. Serum was separated by centrifugation (Refrigerated centrifuge, Hitachi, Japan) at 2500 rpm at 4°C for 15 min. Biochemical parameters such as Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Total bilirubin (TBIL) were determined with commercial kits according to the manufacturer's protocols (Saleem *et al.* 2018).

Histopathological examination

Histopathological evaluation was carried out to spot any changes in liver tissue and cells. The livers obtained from humanely killed mice were washed and kept in 10% v/v buffered formaldehyde (Sigma Aldrich®, Germany). The liver samples were used for the preparation of tissue sections with a microtome and embedded in paraffin wax. Staining was done with hematoxylin and Eosin (H&E) to assess the histopathological changes. Hematoxylin gave blue colour to the nuclei while eosin stained the cytoplasm to pink colour. The slides were studied under light microscope (Meiji Techno Co. Japan) at 10X and 40X magnification (Fatima *et al*., 2019).

STATISTICAL ANALYSIS

Statistical evaluation of hepatoprotective activity was carried out using one-way ANOVA (analysis of variance). The statistical difference in groups was evaluated by Tukey's multiple comparison tests using Graphpad Prism® version 6. The statistical difference was considered significant at $P<0.05$. The results were displayed as Mean ± standard deviation.

RESULTS

Qualitative phytochemical evaluation

The phytochemical analysis of *HSME* showed the presence of flavonoids, proteins, tannins, phenols, saponins, alkaloids, terpenoids, steroids and cardiac glycosides while reducing sugars and carbohydrates were absent in it.

HPLC analysis

Quantitative analysis of *HSME* indicated the presence of various phenol and flavonoids as exhibited in table 1. The HPLC chromatogram of different flavonoid and phenolic compounds are shown in fig. 1 (a,b).The HPLC chromatogram of standard compounds with retention time are shown in fig. 2.

Biochemical parameters

Hepatoprotective activity was evaluated by assessing the serum biomarkers such as AST, ALP, ALT and TBIL in mice pre-treated with 125, 250 and 500mg/kg extract. It was found that the average value of ALT in normal control group (223.8±8.52 IU/l) was significantly lower than paracetamol treated (disease) control group (439.2± 29.05 IU/l). there is decreased in serum enzymes (AST, ALP, ALT and TBI) in group treated with the extract at 125, 250 and 500mg/kg dose as well as silymarin compared to disease control group that was treated with paracetamol only (table 2).

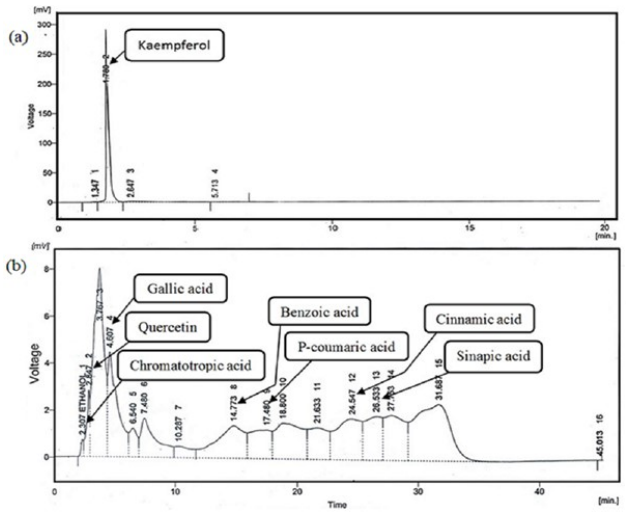

Fig. 1: HPLC chromatogram of aqueous methanolic extract of *Heliotropium strigosum* showing the presence of (a) kaempferol (b) Other flavonoids and phenolic acid

Paracetamol administration did not exhibit a significant increase in ALP level of mice. Pre-treatment with the *HSME* at 250 and 500mg/kg dose decreased the level of ALP in mice. Pre-treatment with the extract also prevented the rise in TBIL in response to paracetamol administration. The changes in liver biochemical parameters of mice in different groups are summarized in table 2.

Acute toxicity study

Albino mice subjected to acute toxicity study showed no sign of toxicity or mortality at 2000 mg/kg. The respiratory rate, heart rate, muscle movements, behaviour, salivation and lacrimation all were normal in treated group. There was no convulsion, abdominal writhes and aggressiveness recorded in treated animals at HSME 2000mg/kg. These observations suggested that the administration of HSME was safe at the highest dose tested (2000 mg/kg).

Histopathological examination

Histopathological evaluation of untreated mice showed normal hepatic parenchyma. Mild lobular inflammation was seen in this group whereas portal inflammation, swelling, steatosis and spotty necrosis were absolutely absent. The disease control group showed severe hepatocellular damage. Moderate portal and lobular

Table 1: Phytochemical components of *Heliotropium strigosum* aqueous methanolic extract with their retention time, area and quantity by HPLC

Compound	Retention time	Area (%)	Quantity (ppm)
Kaempferol	1.780	93.9	628.37
Chromatotropic acid	2.307	0.3	3.8
Quercetin	2.847	1.7	2.61
Gallic acid	4.607	8.6	9.08
Benzoic acid	14.773	7.6	23.53
p-coumaric acid	17.480	4.9	1.87
Cinnamic acid	24.547	8.6	8.75
Sinapic acid	26.533	6.2	2.33

Table 2: Hepatoprotective effects of *Heliotropium strigosum* extract on liver function parameters in mice

Groups	ALT (IU/l)	AST (IU/l)	ALP (IU/l)	TBIL (mg/dl)
Normal control	223.8 ± 8.52	326.8 ± 29.44	281.6 ± 30.10	0.5520 ± 0.02588
Disease control	439.2 ± 29.05 [a] (96.86%)↑$_n$	445.2 ± 33.25 [a] (36.5%) ↑$_n$	326.2 ± 15.99 [a] (16%) ↑$_n$	0.6760 ± 0.03647 [a] (22.4%) ↑$_n$
Silymarin (100mg/kg)	272.8 ± 49.76* (38%) ↓	239.8 ± 22.53* (46.2%) ↓	192.6 ± 13.09* (41.1%) ↓	0.4160 ± 0.02966* (38.3%) ↓
HSME 125 mg/kg	365.0 ± 44.14 (16.8%) ↓	330.2 ± 27.23* (25.8%) ↓	285.0 ± 19.82 (41.1%) ↓	0.5420 ± 0.06496* (18.3%)↓
HSME 250 mg/kg	337.8 ± 47.71* (23.2%) ↓	310.2 ± 24.16* (30.3%) ↓	235.8 ± 44.12* (27.9%) ↓	0.4980 ± 0.03962* (26.3%) ↓
HSME 500 mg/kg	177.8 ± 59.89* (59.6%) ↓	270.8 ± 36.21* (39.3%) ↓	207.0 ± 24.18* (36.5%) ↓	0.4540 ± 0.02608* (32.8%) ↓

*p<0.05 as compared to the disease control; a =significantly different from normal control; n = nonsignificant

Table 3: Histopathological changes in mice liver associated with paracetamol administration with and without pre-treatment with *H. strigosum* extract

Groups	Portal inflammation	Lobular inflammation	Swelling	Fatty change	Spotty Necrosis	Geographic Necrosis
Normal control	-	+ (L,N)	-	-	-	-
Paracetamol Control	++ (L,N)	++ (L,N)	+	+	++	+++
Silymarin 100mg/kg	+ (L,N)	+ (L,N)	-	-	-	-
HSME 125mg/kg	++ (L,N)	++ (L,N)	+	+	++	++
HSME 250mg/kg	++ (L,N)	++ (L,N)	+	+	+	-
HSME 500mg/kg	+ (L,N)	+ (L,N)	+	-	-	-

Where +: Mild, ++: Moderate, +++: Severe, L: Lymphocyte, N: Neutrophil, H&E: Hematoxylin and Eosin

inflammation were also observed in this group. Necrosis, mild swelling and steatosis along with moderate spotty and geographic hepatitis were evident in paracetamol control mice. Silymarin pre-treated group showed mild portal and lobular inflammation whereas swelling, steatosis, geographic and spotty necrosis were not found in this group (fig. 3 and table 3).

Microphotographs of mice liver pre-treated with 125 mg/kg extract showed inflammation, moderate portal and lobular inflammation, mild steatosis and moderate spotty and geographic necrosis. Microphotographs of mice liver pre-treated with 250mg/kg extract also showed spotty necrosis, swelling and inflammation of hepatocytes. Moderate lobular and portal inflammation was also seen whereas geographic necrosis was completely absent (fig. 3; table 3). The slides of mice liver pre-treated with 500 mg/kg extract showed. No necrosis, steatosis and swelling of hepatocytes. Mild portal and lobular inflammation were observed in this group fig. 3.

DISCUSSION

In present study, HSME was evaluated for the phytochemicals through preliminary detection tests. Phenolic and flavonoid compounds were quantified by HPLC. Furthermore, the HSME was evaluated for hepatoprotective effect in mice against paracetamol intoxication at three dose levels. This study revealed the presence of flavonoids, proteins, tannins and phenols, steroids, terpenoids, alkaloids, saponins, steroid ring and cardiac glycoside. Reducing sugars and carbohydrates

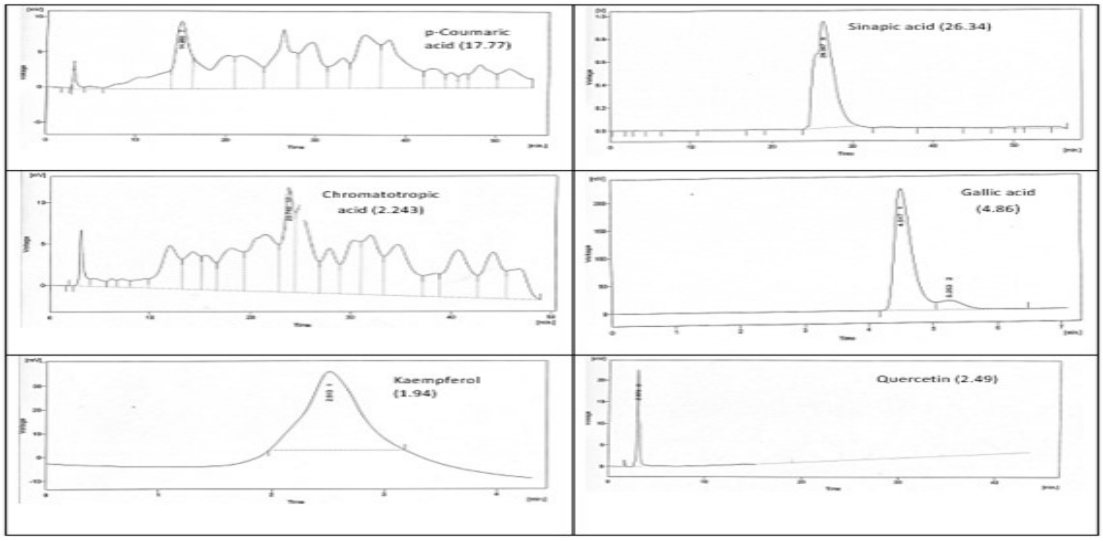

Fig. 2: HPLC chromatogram of standard compounds with retention time

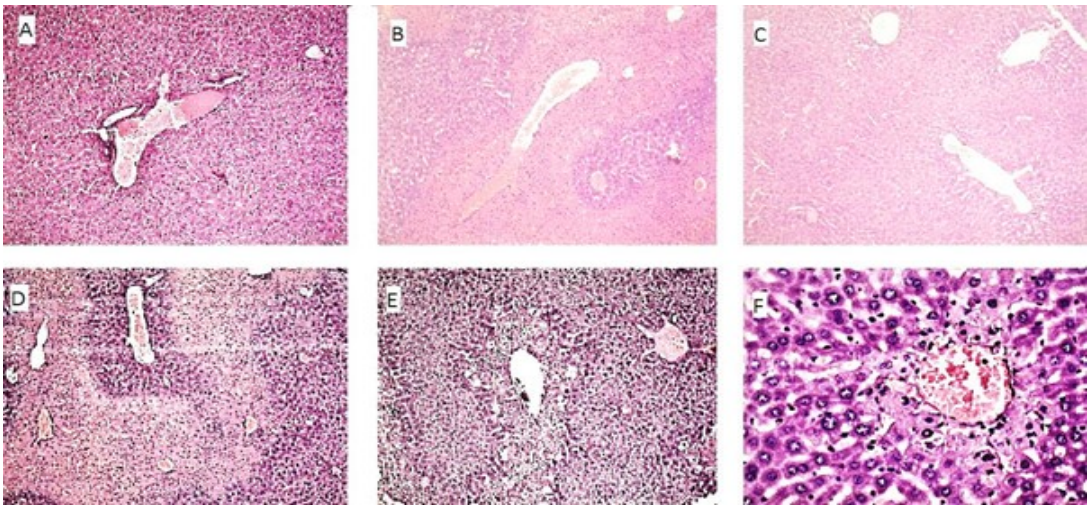

Fig. 3: (A) Microphotograph normal control liver parenchyma at 10x magnification (B) Disease control liver showing geographic necrosis, inflammation and hepatocyte damage (C) Silymarin pre-treated mice liver showing hepatic parenchyma, mild portal and lobular inflammation (D) Mice liver pre-treated with 125mg/kg *HSME* showing geographic necrosis and inflammation as a result of nephrotoxicity from paracetamol. (E) Mice liver pre-treated with 250 mg/kg HSME showing Spotty necrosis, hepatocyte swelling and inflammation can be seen. (F) Liver pre-treated with 500 mg/kg *HSME* showing hepatoprotective effects as necrosis, fatty changes or inflammation of hepatocytes was not seen.

were absent in the *HSME*. These results were also supported by a previous report showing the presence of similar phytochemicals (Saleem *et al.,* 2018).

Therapeutically paracetamol induced hepatotoxicity is the most common cause of fulminant hepatic failure in the UK and other developed countries (Saeed *et al.,* 2017). Paracetamol is metabolised in hepatocyte by cytochrome P450 into N-acetyl p-benzoquinone imine (NAPQI) which is detoxified by glutathione-S- transferase and excreted as mercupturic acid. At high dose, excess of NAPQI is formed which is not detoxified and result in glutathione depletion and decrease in ATP synthesis. This NAPQI excess also impair DNA, RNA and protein structure leading to cell death and necrosis. Reactive oxygen species and oxidative stress are usually implicated in paracetamol induced hepatotoxicity due to excess of NAPQI and interacellular peroxides and depletion of glutathione (Saleem *et al*., 2018). Administration of paracetamol was observed to elevate the average value of AST in mice compared to normal control group. The *HSME* prevented the rise in liver biochemical markers AST, ALP, ALT and TBIL in paracetamol treated mice in dose dependently with the most significant effect observed at 500 mg/kg dose. It was further authenticated by histopathology of the liver. The hepatoprotective effect

of the *HSME* was also remarkably comparable to the standard silymarin therapy. *HSME* strengthen the endogenous antioxidant that assisted in recovery of hepatocyte structure as compared to the disease control group in which live damage due to increase ROS and oxidative stress. Similar mechanism of hepatoprotection has been exhibited by *Azadireachta indica* leaf extract (Chattopadhyay, 2003). Pre-treatment with silymarin prevented the paracetamol induced hepatocellular damage. The *HSME* did not show considerable hepatic protection at 125 mg/kg dose. Other medicinal plants of genus *Heliotropium*, such as *H. foertherianum*, have also shown hepatoprotective potential (Rossi *et al.,* 2012).

Previous investigations have established the hepatoprotective activity of quercetin, gallic acid and kaempferol in animal studies (Chattopadhyay, 2003). Quercetin prevents hepatocellular injury via reduction of oxidative stress (Janbaz *et al*., 2015). Kaempferol and gallic acid also attenuate oxidative stress and lipid peroxidation via antioxidant protection mechanism (Saleem *et al.,* 2018). Previous studies have also shown the antioxidant activity of *H. strigosum* extracts (Shah *et al.,* 2014). The HPLC analysis of the *HSME* indicated the presence of gallic acid, kaempferol, quercetin and other phenolic and flavonoid compounds that might be successively contributed in abating paracetamol induced hepatotoxicity in mice. In addition, a substantial hepatoprotection was presented by *HSME* 500 mg/kg dose due to enormous amount of phenolic and flavnoids in contrast to 250 and 125 mg/kg doses. As *in vitro* antioxidant compounds may exhibit *in vivo* antioxidant activity as well, so this study suggests the role of antioxidant phenolic and flavonoid compounds in hepatoprotective activity of *H. strigosum* extracts (Fatima *et al.,* 2019).

CONCLUSION

It was concluded that *H. strigosum* extract have shown significant hepatoprotective activity in mice against paracetamol intoxication in dose dependent manner. Moreover, the presence of quercetin, gallic acid, kaempferol and other phytochemicals in HSME suggested the probable role of phenolic and flavonoid compounds in protecting paracetamol induced hepatotoxicity. There is need to sort out molecular targets that are key participant of hepatoprotection in furture.

REFERENCES

Basak M and Dey BK (2016). Phytochemical and antipyretic potential of ethanolic leaf extract of Heliotropium indicum L. *J. App. Pharma. Res.,* **4**(2):6-10.

Bjornsson ES (2015). Drug-induced liver injury: An overview over the most critical compounds. *Arch. Toxicol.,* **89**(2): 327-334.

Chattopadhyay R (2003). Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: part II. *J. Ethnopharmacol*., **89**(2-3): 217-229.

Fatima S, Akhtar MF, Ashraf KM, Sharif A and Saleem A (2019). Antioxidant and alpha amylase inhibitory activities of *Fumaria officinalis* and its antidiabetic potential against alloxan induced diabetes. *Cell. Mol. Biol.,* **65**(2): 50-57.

Ghori MK, Ghaffari MA, Hussain SN, Manzoor M, Aziz M and Sarwer W (2016). Ethnopharmacological, phytochemical and pharmacognostic potential of genus Heliotropium L. *Turk. J. Pharm. Sci.,* **13**(1): 143-68.

Gouda AM and Abdelazeem AH (2016). An integrated overview on pyrrolizines as potential anti-inflammatory, analgesic and antipyretic agents. *Euro. J. Med. Chem.,* **114**(2): 257-292.

Janbaz KH, Javed S, Saqib F, Imran I, Zia-Ul-Haq M and De-Feo V (2015). Validation of ethnopharmacological uses of *Heliotropium strigosum* Willd. as spasmolytic, bronchodilator and vasorelaxant remedy. *BMC Complement. Altern. Med.,* **15**(3): 169-179.

Khurm M, Chaudhry BA, Uzair M and Janbaz KH (2016). Antimicrobial, Cytotoxic, Phytotoxic and Antioxidant Potential of *Heliotropium strigosum*. *Willd Medicines.,* **3**(5): 20-28.

Rossi F, Jullian V, Pawlowiez R, Kumar-Roiné S, Haddad M and Darius HT (2012). Protective effect of *Heliotropium foertherianum* (Boraginaceae) folk remedy and its active compound, rosmarinic acid, against a Pacific ciguatoxin. *J. Ethnopharma.,* **143**(2): 33-40.

Saeed A, Wahid F, Rasheed HM, Qayyum R, Shah AJ and Khan T (2017). Effects of *Heliotropium strigosum* and *Trapa bicornis* in hyperactive gut disorders. *Bangladesh. J. Pharmacol.,* **12**(2): 10-17.

Saleem M, Asif A, Akhtar MF and Saleem A (2018). Hepatoprotective potential and chemical characterization of *Artocarpus lakoocha* fruit extract. *Bangladesh J. Pharmacol.,* **13**(1): 90-97.

Saleem A, Saleem M and Akhtar MF (2020). Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. *S. Afr. J. Bot.,* **128**(1): 246-56.

Saleh IA, Vinatoru M, Mason TJ, Abdel-Azim NS, Shams KA and Aboutabl E (2017). Extraction of silymarin from milk thistle (*Silybum marianum*) seeds a comparison of conventional and microwave-assisted extraction methods. *J. Microwave Power Electro. Ener.,* **51**(2): 124-133.

Shah SM, Ullah F, Hussain S, Khan SB, Asiri AM, Ahmad S (2014). A new trypsin inhibitory phthalic acid ester from *Heliotropium strigosum*. *Med. Chem. Res.,* **23**(3): 2712-2714.