



Hepatoprotective and Antibacterial Activity of Ursolic Acid Extracted from *Hedyotis corymbosa* L.

Tamanna Sultana,^{a,*} M. Abdur Rashid,^b M. Ahad Ali^a and Samsuddin Faisal Mahmood^a

^aDepartment of Applied Chemistry and Chemical Technology and ^bDepartment of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.

Abstract

Hedyotis corymbosa (L) (Khetpapra in Bengali), a weedy herb of Rubiaceae family is used as a traditional medicine in Bangladesh for the treatment of Jaundice and other liver disorders. In the present study, hepatoprotective and anti-bacterial activity of ethanolic extract of the herb and ursolic acid isolated from it were investigated against paracetamol induced hepatotoxicity in Long Evan's rats and also against some selective gram positive and ten gram negative bacteria. The ethanolic extract of the plant showed significant hepatoprotective effect as evident by decreased serum enzyme levels, SGPT, SGOT, SAKP and SBIL, and also by normal histological architecture of the liver when compared to the paracetamol treated group. Moreover, ethanolic extract, n-hexane fraction and ursolic acid showed maximum activity of 18, 19, 21 mm against *Sarcina lutea*, *Klebsiella pneumonia* and *Staphylococcus aureus* respectively when compared with standard kanamycin disc (30 µgm/disc). These results thus support *Hedyotis corymbosa* and ursolic acid as hepatoprotective and antibacterial agent.

Key Words: *Hedyotis corymbosa*, Ursolic acid, Hepatoprotective activity, Antibacterial activity.

Introduction

Hepatitis is prevalent throughout the world especially in the tropical and developing countries. It is a common health problem in Bangladesh. In acute hepatitis, normal architecture of the liver drastically collapses due to the damage of hepatocytes and variable numbers of hepatocytes then undergo degeneration or necrosis. Generally, hepatitis has been reported to cause by viruses (hepatic virus A, B, C, D and E etc), non viral infections (toxoplasma, leptospira, icterohaemorrhagiae etc), prolonged drug therapy (paracetamol, sulpho-namides, phenyl benzoate, para amino salicylic acid etc), excessive alcoholism and exposure to toxic chemicals (carbon tetrachloride, thioacetamide, galacto-samine etc (Saraswat *et al.*, 1996). However, the extent of hepato-cellular damage extremely varies due to modifying factors like doses of toxic agents, virulence of infecting organisms and the resistance of the host. At present there is no effective remedy of hepatitis including viral hepatitis C, D and E as well as, the available treatment options are highly expensive.

Bangladesh is a good emporium of medicinal plants. Since time immemorial medicinal plants have been used in the treatment of diverse diseases in this region. *Hedyotis corymbosa* (L) Lam. popularly known as khetpapra in Bengali is a weedy herb of Rubiaceae family, widely distributed through

out Bangladesh especially in the hill areas. Most of the species of Rubiaceae possess medicinal properties and are used by Chinese, Malays and Indians (Sadasivan *et al.*, 2006). In modern Chinese practice *Hedyotis corymbosa* (HC) is extensively used for the treatment of viral infections, syndromes involving toxic heat, cancer, acne, boils, skin ailments, appendicitis, hepatitis, eye diseases and bleeding (Sadasivan *et al.*, 2006; Lin *et al.*, 1987). However, in Bangladesh the extract of this herb is traditionally used for the treatments of jaundice and other liver disorders. This plant is also reported to use for treating venomous bites. The plant extract is bitter, cooling, febrifugal, pectoral, anthelmintic, diuretic, depurative, diaphoretic, expectorant, digestive and has stomachic properties (Kirtikar and Basu, 1994). Biological investigations of khetpapra revealed its activities on skin diseases, bronchitis, constipation, nervous depression caused by deranged bile, vitiated conditions of pitta, hyperdipsia, hepatopathy (Warrier *et al.*, 1995), antitumor (Takagi *et al.*, 1981) and weed control activities (Gilreath and Harbaugh, 1986).

Scientists have shown their interest on the triterpinoid compounds that are major components of foods, some medicinal plants and other plants because of their biological activities. Among these, ursolic acid (3 hydroxy-urs-12en-28-oic acid),

* Corresponding author: E-mail: tsultana_du@yahoo.com

a steroid like pentacyclic acid present in medicinal plants like *Eriobotrya japonica*, *Rosmarinus officinalis*, *Eucalyptus tereticornis* (Saraswat *et al.*, 2000), *Melaleuca leucadendron*, *Ocimum sanctum*, *Glechoma hederaceae* and *Piper betle* (Liu, 1995) in the form of free acid or as aglycone of triterpenoid saponins is an important agent. Ursolic acid isolated from *Eucalyptus tereticornis* has been found to possess antiinflammatory, antiulcer, cytotoxic activities (Saraswat *et al.*, 2000). Liu J (1995) also reported analgesic, antitumor, antiinflammatory and cardiotoxic effects of ursolic acid. Hepatoprotective activity of ursolic acid (isolated from *Eucalyptus tereticornis*) against chemical (thioacetamide, galactosamine and carbon tetrachloride) induced toxicity in rats was evaluated by comparing with a known hepatoprotective drug (silymarin) where three parameters were assessed i.e., trypan blue viability, oxygen consumption and bile tests (Saraswat *et al.*, 1996). In recent times, effects of ursolic acid in Leukemic cells (Ovesna *et al.*, 2006), ethanol induced oxidative stress (Saravanan and Pugalendi, 2006), experimental hypertension (Somova *et al.*, 2003) and ethanol mediated liver damage (Sravanan *et al.*, 2006) were reported using ursolic acid collected from synthetic routes or from natural products. In addition, hepatoprotective activity of *Hedyotis corymbosa* was reported by Sadasivan *et al.*, (2006) on paracetamol induced liver damage; however, report on ursolic acid isolated from the same source for the aforementioned activities remained unpublished.

Therefore, considering the high incidence of hepatitis in Bangladesh and insufficiency of any cheap and effective modern drug, the present study was focused to evaluate ursolic acid extracted from *Hedyotis corymbosa* on paracetamol induced hepatotoxicity in Long Evan's rats by assessing biochemical and histopathological tests, as well as, its antibacterial activity.

Materials and Methods

Extraction

Whole plants of *Hedyotis corymbosa* (HC) were collected from Jahangirnagar University campus, Savar, Dhaka. The powdered plant material (500g) was extracted with 95% ethanol at room temperature for three weeks with shaking and stirring. Filtration followed by evaporation of solvent using a rotary evaporator at low temperature and pressure yielded 5 g of extract.

Fractionation and isolation of ursolic acid

The crude ethanol extract (5 g) of HC was diluted with 100 ml of 90% methanol. The resultant mother solution was subjected to solvent-solvent partition (Rashid *et al.*, 1995;

Buckingham 1982) using n-hexane, carbon tetrachloride and chloroform which yielded 0.5, 2.0 and 1.2 g of extracts respectively. The chloroform soluble fraction (1.2 g) was fractionated by column chromatography on silica gel (60-120 mesh) using hexane-ethyl acetate in different ratio. Fraction eluted with 30% ethyl acetate in hexane gave compound ursolic acid (30 mg), which was re-crystallized with ethanol. The carbon tetrachloride soluble fraction from solvent-solvent partitioning process gave crystals after evaporation of the solvent. These crystals were purified by preparative layer chromatography (PLC) on silica gel and then recrystallization with ethanol also yielded 20 mg of ursolic acid (Sultana *et al.*, 1999).

Experimental design for hepatotoxicity and hepatoprotective activity study

20 Long Evans strain of Norwegian rats were collected from Animal Resources Branch of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B) for this study. The rats were 70-80 days old and body weight ranged from 170 to 225 gm. All the rats were kept in a clean animal house at the Department of Pharmacy, University of Dhaka under standard laboratory conditions. Each rat was fed with 20 gm of commercial 'rat pallet' supplied by ICDDR'B per day. All animal experiments were carried out according to Bangladesh Medical Research Council guidelines. The experiment was designed with five groups (A-E) each containing 4 rats except group C (6 rats). The experimental groups were:

- * Control group- Group A: Rats were fed with normal rat pallets.
- * Vehicle control group- Group B: Rats were injected intraperitoneally with vehicle (propylene glycol) at a single dose of 9 ml/Kg body wt.
- * Paracetamol plus vehicle treated group- Group C: Rats were injected with paracetamol plus propylene glycol solution (72.5 mg/ml) intraperitoneally, as a single dose of 2 gm/kg body wt (Mitra *et al.*, 1998).
- * Crude HC extract plus paracetamol treated group- Group D: Rats were administered with 200 mg crude HC extract (15.26 mg/ml)/kg body wt. orally by a plastic stomach tube, once daily up to 7 days. On 7th day along with the plant extract, paracetamol (in propylene glycol, 110.3 mg/ml) was also injected intraperitoneally as a single dose of 2 gm/kg body wt.

- * Ursolic acid plus paracetamol treated group- Group E: Rats were administered with 5 mg ursolic acid (0.75 mg/ml)/kg body weight orally once daily up to 7 days. On 7th day along with this oral dose, paracetamol (110.3 mg/ml) was also injected intraperitoneally as a single dose of 2 gm/kg body wt.

The rats were kept in fasting condition overnight before the test was performed. The animals were sacrificed 48 hours after paracetamol or specific dose (for a particular group) administration with mild ether anesthesia.

Assessment of liver functions

After sacrifice, blood samples from each group of rats were collected in centrifuge tubes, allowed to clot at room temperature and the serums were separated by centrifugation (3000 rpm, 15 minutes). Serum samples were subjected to liver function tests of serum enzymes such as serum glutamate-pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), serum glutamate-oxaloacetate transaminase (SGOT) (Reitman and Frankel, 1957), serum alkaline phosphatase (SAKP) (Bessay *et al.*, 1956) and serum bilirubin (SBIL) (Malloy *et al.*, 1937) by Standard enzymatic colorimetric method at N. K. Al-Amin Diagnostic Centre, Dhaka, Bangladesh.

Histopathological study

Liver specimens of rats were sliced (few mm thickness) and fixed in 10 % buffered formalin for three days. The tissues were dehydrated in ethanol, cleaned in xylene, embedding in paraffin, sectioned (6 μ thickness) by rotating microtome at 6 micron thickness and placed on glass slides pretreated with egg albumin solution. The sections were deparaffinized by xylene, hydrated in alcohol, washed with water and kept in Harris haematoxylin solution. Glass slides containing the tissues were finally cleaned in xylene, wiped, dried and then a drop of D. P. X. mounting fluid was introduced on the sections. Liver histological architecture was examined with the help of photomicroscope in Armed Forces Institute of Pathology and Transfusion (AFIP and T) department, Combined Military Hospital (CMH), Dhaka, Bangladesh.

Antibacterial study

The antibacterial activity of the crude ethanolic extract of *Hedyotis corymbosa* (500 μ g / 10 μ l of chloroform), n-hexane fraction (500 μ g / 10 μ l of chloroform) and isolated ursolic acid (100 μ g / 10 μ l of chloroform) were tested against 4 gram positive (*Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea* and *Staphylococcus aureus*) and 10 gram negative (*Shigella sonnei*, *Shigella dysenteriae*, *Shigella boydii*,

Salmonella para typhi A, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Vibrio cholerae* and *Klebsiella pneumonia*) bacteria. Pure cultures of these bacterial strains were taken from the Microbiological Laboratory of the Institute of Nutrition and Food Sciences (INFS), University of Dhaka and also from ICDDR'B. The study was carried out by the disc diffusion technique (Bauer *et al.*, 1966) where standard Kanamycin disc (30 μ g of Kanamycin/disc, Mast Diagnostic, Merseyside, UK) was used to compare the results. The discs were prepared by filter papers (6 mm diameter) and then autoclaved at 121 $^{\circ}$ C for 15 minutes. Sample and standard impregnated discs were placed gently no closer than 15 mm to the edge of the solidified agar plate freshly seeded with the test organism and ensured that the discs were far apart enough to prevent overlapping the zones of inhibition. The plates were refrigerated for 24 hours at 4 $^{\circ}$ C and then incubated at 37 $^{\circ}$ C for 12-18 hours. Antibacterial activities of the samples were expressed by measuring the zones of inhibition in millimeter.

Statistical analysis

For the hepatoprotective study the biochemical parameters obtained from each group of rats were statistically analyzed by independent sample t-test using SPSS 13.0 (2004). The mean values with standard error, degree of freedom, t-values and probability were illustrated in Table I.

Results and Discussion

Chromatographic separation of the ethanol extract afforded a pure compound, the structure of which was established as ursolic acid by careful analysis of the spectroscopic data, including H-NMR and MS and was reported by Sultana *et al.*, (1999).

Hepatoprotective activity

The crude ethanol extract of HC and ursolic acid were tested for hepatoprotective activity by measuring and comparing the biochemical parameters like serum enzymes SGOT, SGPT, SAKP, SBIL among the different groups of rats (Table I) and also through histological examinations (Fig. 1 to 3).

At first, the control group A was compared with the vehicle control group B which showed non significant variations ($P = 0.276$ to 1.0 , $df = 6$) for all the biochemical parameters tested (SGOT, SGPT, SAKP, SBIL) in this experiment (Table-I). However, the comparison of group A with paracetamol plus vehicle treated group C showed significant increase in all serum hepatic enzyme levels investigated, at P values ranged from 0.035 to 0.000 ($df = 8$). For instance,

Table I. Comparisons of serum marker hepatic enzymes among different groups of rats.

Bio-chemical parameters (hepatic enzymes)	Groups of rats	Average body weight of rats (gm) (n=4)	Mean value for bio-chemical parameters M \pm SEM (n=4)	Comparisons	t value	Degree of freedom(df)	P value
SGOT U/L	A	203.25	12.00 \pm 1.472				
	B	161.25	12.00 \pm 2.041	A vs B	0.00	6	0.500, NS
	C	158.00	16.17 \pm 1.424	A vs C	-1.96	8	0.043*
	D	177.00	11.75 \pm 0.629	C vs D	2.39	8	0.022*
	E	183.25	11.50 \pm 0.646	C vs E	2.52	8	0.018*
SGPT U/L	A	203.25	14.25 \pm 1.931				
	B	161.25	12.75 \pm 1.797	A vs B	0.57	6	0.295, NS
	C	158.00	27.5 \pm 0.671	A vs C	-7.61	8	0.000***
	D	177.00	13.5 \pm 0.646	C vs D	14.26	8	0.000***
	E	183.25	14.5 \pm 0.289	C vs E	14.96	8	0.000***
SAKP U/L	A	203.25	39.25 \pm 4.715				
	B	161.25	45.50 \pm 2.217	A vs B	-1.20	6	0.138, NS
	C	158.00	54.84 \pm 1.797	A vs C	-3.58	8	0.004**
	D	177.00	41.00 \pm 1.291	C vs D	5.61	8	0.001***
	E	183.25	41.25 \pm 1.493	C vs E	5.34	8	0.001***
SBIL mg%	A	203.25	1.35 \pm 0.155				
	B	161.25	1.58 \pm 0.307	A vs B	-0.66	6	0.269, NS
	C	158.00	2.58 \pm 0.465	A vs C	-2.08	8	0.036*
	D	177.00	1.20 \pm 0.082	C vs D	2.37	8	0.023*
	E	183.25	1.40 \pm 0.041	C vs E	2.03	8	0.039*

n = Number of replicates, M=Mean value, SEM= Standard Error of Means, NS = Not significant, P = Probability, df = Degree of freedom, * = Significant at P < 0.05, ** = Significant at P < 0.01, *** = Significant at P < 0.001, A = Control group, B = Vehicle Control group, C = Paracetamol plus vehicle treated group, D = Crude *Hedyotis corymbosa* extract plus paracetamol treated group, E = Ursolic acid plus paracetamol treated group, SGOT = serum glutamate-oxaloacetate transaminase, SGPT = serum glutamate-pyruvate transaminase, SAKP = serum alkaline phosphatase, SBIL = serum bilirubin, vs = versus

SGOT, SGPT, SAKP, SBIL values increased by 34.75, 92.98, 39.72 and 9.11% respectively when group A was compared with group C and this observation was the obvious sign of leaking of cellular enzyme into the plasma which consequently indicated the liver injury in rats of group C. However, vehicle treated group B itself showed no significant effect in serum enzymes. Therefore, rats treated with over dose of paracetamol developed significant hepatic damage (Group C, 2gm/kg body wt), which was observed through substantial increase in the concentration of serum parameters. This observation was strengthened by histopathological observations (Fig. 1) of the liver slides. In group A, the histological findings showed no pathological changes i.e., no inflammatory cells, no vacuolation in the cytoplasm of hepatocytes, no regeneration of hepatocytes,

no congestion in sinusoids and no necrosis (Fig. 1, slide A). Similarly, group B showed identical characteristics (Figure 1, slide B) with group A, which confirmed that propylene glycol used in group B did not contribute to produce hepatotoxicity. Whereas, in paracetamol plus vehicle treated group C marked histological changes such as centrilobular necrosis with mononuclear inflammatory cell, vacuolation in cytoplasm of hepatocytes, ballooning degeneration and severe congestion in sinusoids were found (Fig. 1, slides C-F). All these histological findings in addition of biochemical changes confirmed that paracetamol drug at a dose of 2 gm/kg body wt, intraperitoneally, successfully produced severe hepatotoxicity in rats of group C.

Pretreatment of the rats with crude ethanol extract of HC (Group D) at 200 mg/kg body wt. and ursolic acid (Group E)

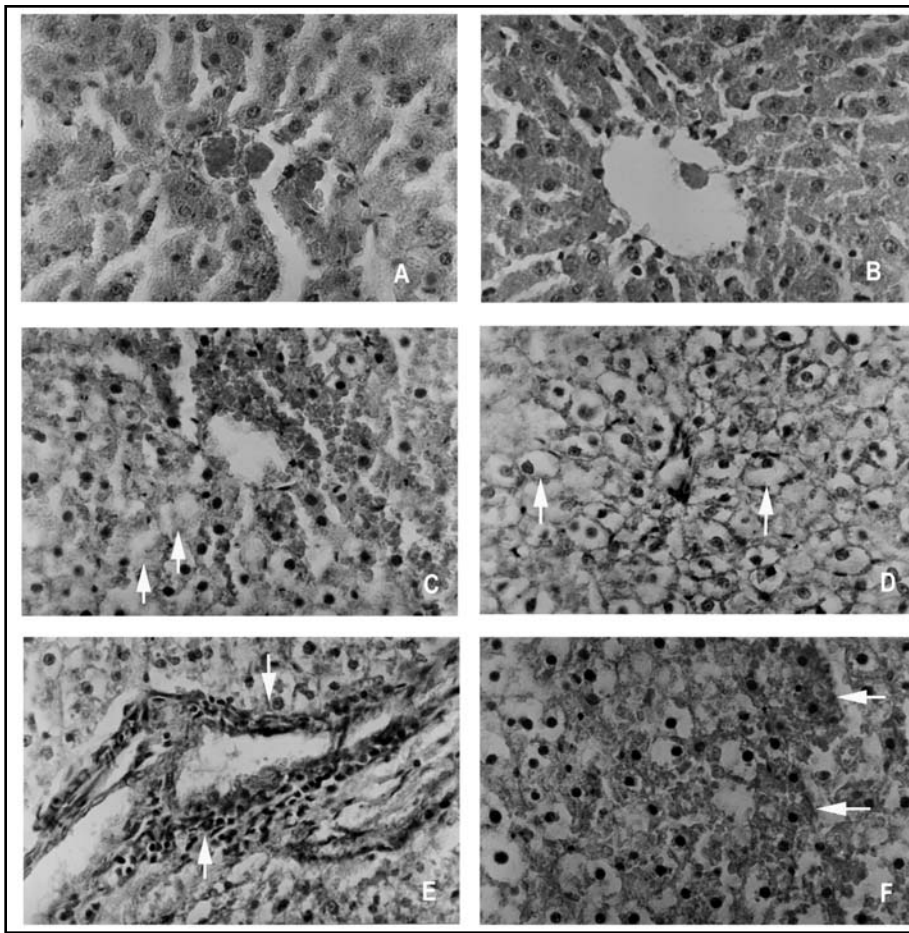


Fig. 1. Characteristic histopathological observations noticed in liver specimens of Group A, B and C rats

Liver slides A and B of Group A and Group B respectively showed normal liver architecture of rats whereas, four liver slides (C-F) of Group C rats (treated with paracetamol plus propylene glycol solution at 2 gm/kg body wt of rat, single dose) showed abnormal architecture of liver e.g. centrilobular necrosis marked in Slide C, Ballooning degeneration in Slide D, inflammatory cells in Slide E and congestion in sinusoids in Slide F.

at 5 mg/kg body wt. for 7 days prior to paracetamol administration caused a significant reduction in serum enzymes (Table I). For instance, 27.33, 50.9, 25.23 and 53.48% decrease in SGOT, SGPT, SAKP, SBIL levels respectively were found when compared between paracetamol (group C) versus HC treated (D) groups which indicated hepatoprotective potentiality of HC. Similarly, ursolic acid treated group significantly reduced 28.88, 47.27, 24.78 and 45.73% of SGOT, SGPT, SAKP and SBIL levels respectively in blood serum when compared with group C (Table I). Therefore, pretreatment of rats with extract of HC as well as, herb component-ursolic acid for 7 days prior to paracetamol administration resulted in the significant protection of paracetamol induced elevation of serum marker hepatic enzymes. Thus,

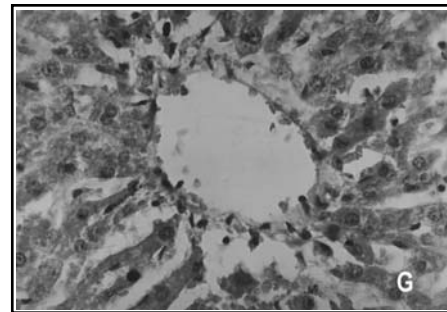


Fig. 2. Characteristics histopathological observations found in liver architecture of Group D rats

Liver specimen (slide G) of group D rats (treated with 200 mg crude HC extract once daily for 7 days and on 7th day paracetamol was injected at 2 mg/kg body wt and then sacrificed after 48 hours) showed normal liver characteristics e.g. no necrosis, no vacuolation in the cytoplasm, mild inflammatory cells and mild sinusoidal congestion and regeneration

both crude ethanol extract of HC and ursolic acid appeared to be effective in reducing the injurious effect of paracetamol noticed in this study. Moreover, the histopathological findings for HC extract plus paracetamol treated group D (Figure 2) and also for ursolic acid plus paracetamol treated group E (Figure 3) showed normal liver characteristics i.e. absence of necrosis and vacuolation of cytoplasm etc.

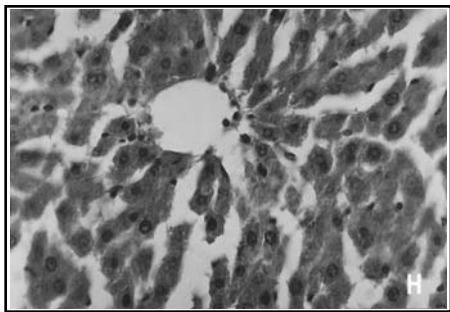


Fig. 3. Characteristics histopathological observations of liver specimen of Group E rats

Liver specimen (slide H) of group E rats (treated with 5 mg ursolic acid once daily for 7 days and on 7th day paracetamol was injected at 2 mg/kg body wt and then sacrificed after 48 hours) showed normal liver characteristics e.g. no necrosis, no vacuolation in the cytoplasm, no inflammatory cells and no sinusoidal congestion but mild regeneration.

Antibacterial activity

The crude ethanol extract of HC showed a significant antibacterial activity specially against one gram positive (*Sarcina lutea*) and two gram negative bacteria (*Shigella sonnei*, *Vibrio cholerae*). The zones of inhibition observed for *Sarcina lutea*, *Shigella sonnei*, *Vibrio cholerae* were 18, 15 and 14 mm respectively where 500 µg of ethanol extract of HC/disc was compared with standard Kanamycin zones (30, 30, 33 mm) at concentration of 30 µg/disc. The n-hexane fraction of the plant HC at concentration of 500 µg/disc showed maximum activity against *Klebsiella pneumonia* (19 mm) and moderate activity against *Sarcina lutea* (16 mm), *Shigella flexneri* (10 mm). In contrast, isolated ursolic acid (100 µg/disc) showed significant activity against *Staphylococcus aureus* (21 mm) and *Shigella boydii* (19 mm) and moderate activity against *Pseudomonas aeruginosa* (10 mm) when compared with standard Kanamycin zones. Mild activity (inhibition zones 6-10 mm) noticed for rest of the microorganisms used in this experiment. As the antibacterial activity of HC extract and ursolic acid were

found different it seems that other active constituents may also responsible for the characteristic inhibition.

Paracetamol is a well known anti-pyretic and analgesic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (Mitchell *et al.*, 1973). Most of the hepatotoxic chemicals including paracetamol damaged liver mainly by inducing lipid peroxidation directly or indirectly (Sadasivan *et al.*, 2006). In higher animals, lipid peroxidation was known to cause destabilization and disintegration of the cell membrane, leading to liver injury, arteriosclerosis and kidney damage (Chang *et al.*, 1994). Peroxy radicals are important agents that mediate lipid peroxidation thereby damaging cell membrane. Generally, when liver plasma cell is damaged, due to the disturbance caused in transport functions of hepatocytes (Zimmerman *et al.*, 1970), a variety of enzymes located normally in cytosol is released into the blood which causes increased enzyme levels in the serum. Therefore, the estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Sadasivan *et al.*, 2006). Thus, in the present study a marked increase in hepatic enzymes i.e. SGOT, SGPT, SAKP and SBIL in group C rats by administration of paracetamol (2g/kg body wt) indicated the potentiality of paracetamol as a hepatotoxic agent, which was supported by the observations of Sadasivan *et al.* (2006) and Torrielli (1978). Lin *et al.* (2004) reported HC as an antilipid peroxidant, at a concentration of 10 mg/ml, since HC inhibited the end product of lipid peroxidation (the malondialdehyde, MDA) strongly. Moreover, Sadasivan *et al.* (2006) proposed that HC stimulated hepatic drug metabolizing enzymes through reduction of duration of hexobarbitone-induced necrosis. HC also reported to contain nine iridoid glycoside derivatives (Otsuka *et al.*, 1991) and iridoid glycoside from *Picrorrhiza kurroa* showed significant protective action on liver against carbon tetrachloride intoxicated rats. HC also reported to contain oleanolic acid, triterpinoid and γ sitosterol which are addressed to increase antioxidant components in the liver. Thabrew *et al.* (1987) reported that the serum level of transaminase returns to normal levels with healing of parenchyma and the regeneration of hepatocytes. Further the stimulation of hepatic regeneration was known to make the liver more resistant to damage by toxins (Lesch *et al.*, 1970). Therefore, though paracetamol was injected with crude HC extract or ursolic acid treated groups (D and E respectively) at the same dose (2mg/kg) that produced hepatotoxicity in group C, but did not produce any hepatotoxicity due to the

protective roles of the crude extract as well as, the extracted ursolic acid from that plant. This observation was indicated by significant reduction of serum hepatic enzyme levels (Table I) and also by normal liver characteristic with mild regeneration found in liver specimens of group D (Fig. 2) and E (Fig. 3). Preventing liver lesions from progressing to fibrosis and cirrhosis, and repairing parenchymal cell damage by stimulating liver regeneration are important mechanisms for hepatoprotection. The triterpenoid ursolic acid and perhaps the iridoid glycosides present in HC seems responsible for the marked hepatoprotective effects, observed in the present study. Therefore, it may be suggested that both HC extract and its component-ursolic acid act by their stabilizing effect on the plasma membrane as was reported in the case of Silymarin (Ramellini and Meldolesi, 1976). Further, the stimulation of hepatic regeneration makes the liver more resistant to damage by the toxin. Therefore, it seems HC and ursolic acid possessed liver lipid peroxidation inhibition and resultant tissue degeneration and thus showed potential hepatoprotective action upon paracetamol induced hepatic damage in rats.

The antioxidant study by Ahmad *et al.*, (2005) suggested that *Hedyotis herbacea* contains constituents that are good radical scavengers while the other *Hedyotis* species contain constituents that inhibit oxidation through a mechanism other than radical-scavenging. They also stated that the significant inhibitory activity of root extracts of *Hedyotis* species (*H. capitellata* and *H. dichotoma*) against the mutant (DNA repair deficient) compared to the wild strains of *B. subtilis* suggested a possible involvement of DNA inhibition mechanism. Whereas, Tomlinson and Palombo, (2005) found that *E. duttonii* extract was membrane active against *S. aureus*. However, exact mechanisms of antibacterial activity of *Hedyotis* species or triterpenoid ursolic acid constituents are not found in the literature. In this study bacterial growth disturbance was noticed for few test microorganisms. However, two parts of the whole research, hepatoprotective and antibacterial activity studies, were not related and were two completely independent events. Therefore, further investigation could be worthwhile to explain the exact protective mechanisms of *Hedyotis corymbosa* and ursolic acid against hepatitis and bacterial infections

Acknowledgement

The authors are grateful to the authority of N. K. Al Amin diagnostic centre and AFIP&T of CMH Dhaka Cantonment for testing biochemical parameters and histopathological examinations respectively and Dr. F. Kiuchi, Faculty of

Pharmaceutical sciences, Kanazawa University, Japan for the spectral data of ursolic acid.

References

- Ahmad R., Ali A. M., Israf D. A., Ismail N. H, Shaari K. and Lajis N. H. (2005). Antioxidant, radical-scavenging, antiinflammatory, cytotoxic and antibacterial activities of methanolic extracts of some *Hedyotis* species. *Life Sci.* **76**: 1953-64.
- Bauer A. W., Kibby W. M. M., Sherries J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**: 493-496.
- Bessay O. A., Lowry O. H. and Brock M. T. (1956). A method for the rapid determination of alkaline phosphate with five cubic millimeters of serum. *J. Bio. Chem.* **164**: 321.
- Buckingham J. (1982). Dictionary of Organic Compounds. Vol. V. 5th Edition. D-5676.
- Chang C. H., Lin C. C., Htoori M. and Namba T. (1994). Effects of rosemary extracts and major constituents on lipid oxidation and soyabean lipoxygenase activity. *J. Am. Oil. Chem. Soc.* **69**: 999-1002.
- Gilreath J. P. and Harbaugh B. K. (1986). Chemical weed control in caladiums grown in organic soil. *Proc. Fla. Hort. Soc.* **98**: 107-110.
- Kirtikar K. R. and Basu B. D. (1994). Indian Medicinal Plants. Edited by B Singh MP Singh, 2nd Revised Edition, **2**:1263.
- Lesch R., Reutter W., Keppler D. and Decker K. (1972). Liver restitution after acute galactosamine hepatitis: auto-radiographic and biochemical studies in rats. *Exp. Mole. Path.* **12**: 58-69.
- Lin C. C., Chen F. Y. and Namba T. (1987). Development of crude drug resources from Taiwn, pharmaco-gnostical studies on a Chinese crude drug. *Shoyakugaku Zasshi.* **41**:180-188.
- Lin C. C., Ng L. T. and Yang J. J. (2004). Antioxidant activity of extracts of "Peh- Hue-Juwa-Chi-Cao" in a cell free system. *Am. J. Ch. Med.* **32**: 339-349.
- Liu J. (1995). Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacol.* **49**: 57-68.

- Malloy H. T. and Evelyn K. A. (1937). The determination of bilirubin with the photometric colorimeter. *J. Bio. Chem.* **119**: 481-490.
- Mitchell J. R., Jollow D. J., Potter W. Z., Gillette J. R. and Brrodle B. N. (1973). Acetaminophen induced hepatic necrosis. I. Role of drug metabolism. *J. Pharmacol. Exp. Therap.* **187**: 185-194.
- Mitra S. K., Venkataranganna M. V., Sundaram K. and Gopumadhavan, S. (1998). Protective effect of HD-03, a herbal formulation against various hepatotoxic agents in rats. *J. Ethnopharmacol.* **63**: 181-186.
- Otsuka H., Yoshimura K., Yamasaki K. and Cantoria M. C. (1991). Isolation of acyl iridoid glycosides from a Philippine medicinal plant *Oldenlandia corymbosa*. *Chem. & Pharm. Bull.* **39**: 2049-2052.
- Ovesna Z. and Kozics K. Slameaová. (2006). Protecting effects of ursolic acid and oleanolic acid in leukemic cells. *Fun. Mol. Mech. Mutagen.* **600**: 131-137.
- Ramellini G. and Meldolesi J. (1976). Liver protection by Silymarin. *In vitro* effect on dissociated rat hepatocytes. *Arzneimittel Forschung (Drug Research)*. **26**: 69-73.
- Rashid M. A., Gustafson K. R., Kashmann Y., Cardellen J. H., Mc Mahon J. B. and Boyd M. R. (1995) Anti-HIV alkaloid from *Toddalia asiatica*. *Nat .Prod. Lett.* **6**: 153-156.
- Reitman S. and Frankel S. (1957). Determination of serum glutamate oxaloacetate and glutamic pyruvic acid transaminases. *Am. J. Clin. Pathol.* **28**: 56-66.
- Sadasivan S., Latha P. G., Sasikumar J. M., Rajashekar S., Shyamal S. and Shine V. J. (2006). Hepato-protective studies on *Hedyotis corymbosa* (L.) Lam. *J. Ethnopharmacol.* **106**: 245-249.
- Saraswat B., Visen P. K. S., Dayal R. and Agarwal D. P. (2000) Ursolic acid isolated from *Eucalyptus tereticornis* protects against ethanol toxicity in isolated rat hepatocytes. *Phytother. Res.* **14**: 163-166.
- Saraswat B., Visen P. K. S., Dayal R., Agarwal D. P. and Patnaik G. K. (1996). Protective action of ursolic acid against chemical induced hepato-toxicity in rats. *Ind. J. Pharmacol.* **28**: 232-239
- Saravanan R. and Pugalendi V. (2006). Impact of ursolic acid on chronic ethanol-induced oxidative stress in the rat heart. *Pharmacol .Rep.* **58**: 41-47.
- Saravanan R., Viswanathan P. and Pugalendi K. V. (2006). Protective effect of ursolic acid on ethanol-mediated experiment-tal liver damage in rats. *Life Sci.* **78**: 713-718.
- Somova L. O., Nadar A. and Rammanan. Shode F. O. (2003). Cardiovascular, antihyperlipide-mic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomed.* **10**: 115-121.
- SPSS 13.0 for windows, Release 13.0 (2004). SPSS Inc.
- Sultana T., Ali M. A. and Rashid M. A. (1999). Isolation and Characterization of ursolic acid from khetpapa (*Hedyotis Corym-bosa*). *Dha .Univ. J. Bio. Sci.* **8**(1): 67-71.
- Takagi S., Yamaki M., Masuda K., Nishihama Y. and Sakina K. (1981). Studies on the herb medical materials used for some tumor II, on the constituents of *Hedyotis corymbosa* Lam. *Shoyakugaku Zasshi.* **101**(7): 657-659.
- Thabrew M. I., Joice P. D. T. M. and Rajatissa, W. A. (1987). Comparative study of the efficacy of *Pavetta indica* and *Osbeckia octandra* in the treatment of liver function. *Planta. Medica.* **53**: 239-241.
- Tomlinson S. and Palombo E. A. (2005). Characterisation of antibacterial Australian medicinal plant extracts by investigation of the mechanism of action and the effect of interfering substances. *J. B. Microbiol.* **45** (5): 363-370.
- Torrielli MV. Pathological aspects of liver injury produced by drugs. In: Slater, TF. editor, *Biochemical Mechanisms of Liver Injury*. London: Academic Press, UK, 1978; p. 631.
- Warrier P. K., Nambiar V. P. K. and Ramankutty C. (1995). *Indian Medicinal Plants- A Compendium of 500 Species*, Chennai: Orient Longman Ltd. **3**: 120-123.
- Zimmerman H. J. and Seeff L. B. (1970). Enzymes in hepatic disease. In: Goodly EL, editor, *Diagnostic Enzymology*. Philadelphia: Lea & Febiger, USA. p. 1-38.

Received : April 27, 2009;

Accepted : July 22, 2009