doi.org/10.36721/PJPS.2021.34.2.SUP.737-745.1

Therapeutic role of *Typha elephantina* leaves aqueous extract in paracetamol intoxicated rabbits

Bashir Ahmad[1], Ali Muhammad Yousafzai[1], Alam Zeb[3], Waqar Ali[3], Nadir Zaman Khan[3], Muhammad Aasim[3], Saeed Ahmad[2], Shariat Ullah[5], Ayaz Ali Khan[3], Farhat Naz[4] and Sumayya Raziq[1]

[1]Department of Zoology, Islamia College, Khyber Pakhtunkhwa, Peshawar, Pakistan
[2]Department of Zoology, Malakand University, Khyber Pakhtunkhwa, Pakistan
[3]Departments of Biotechnology, Malakand University, Khyber Pakhtunkhwa, Pakistan
[4]Department of Zoology, University of Sherengal, warai Campus, Khyber Pakhtunkhwa, Pakistan
[5]Department of Botany, Malakand, University Khyber Pakhtunkhwa, Pakistan

Abstract: Present study is aimed to investigate the hepatoprotective and hematopoietic effect of *Typha elephantina leaves aqueous* (T.E.AQ), extract in paracetamol (PCM) intoxicated rabbits. Experimental animals were divided into various groups. The blood was taken on day 7th (W1=Week 1), day 14th (W2 = week 2) and day 21st (W3 = week 3) of treatments and was analyzed for all hematological and serum biochemical markers. PCM administration caused marked increase in the levels of serum biochemical and hematological parameters. The leaves of T.E.AQ extract at dose rate 300mg/kg body weight significantly ($P<0.05$) reduced the elevated levels of serum biochemical and hematological indices towards normal values on third week (day 21st) of treatment while treatment in the first two weeks revealed non-significant effects even at all doses of extract. The levels of glutathione (GSH) and radical scavenging activity (RSA) were reduced and thiobarbituric acid reactive substances (TBARS) levels was high in the PCM feed animals. Administration of (T.E.AQ) extract at high dose (300mg/kg) significantly regulated and normalized these antioxidant values. The antioxidant capacity of (TE.AQ) extract, showed increase inhibition against various extract concentrations on the basis of percent scavenging of (DPPH) free radical. The histological sections of liver further supported the hepatoprotective activity of extract.

Keywords: *Typha elephantina,* alkaline phosphatase, mean corpuscular hemoglobin, creatinine, hepatoprotective.

INTRODUCTION

Liver is a vital organ in the body, having a key role in regulating various vital processes, occurring in the body, such as metabolism, secretion and storage. Thus provide protection to the body, against toxic materials by the detoxification and excretion of Xenobiotics from the body. As a result of this, the liver is exposed to all types of endogenous and exogenous toxic agents, which may produce (Kirman *et al*., 2020).

Many chemicals and drugs, which are used on a regular basis, result in cellular as well as metabolic liver damage (Weaver *et al*., 2020). Acetaminophen, also known as paracetamol, is one of the well-known analgesic and antipyretic agent that cause hepatotoxicity and nephrotoxicity (McCrae *et al*., 2018). Paracetamol has no toxic effect, when prescribed in a normal dose, but its high dose, result in liver damage. Damage to the liver with the acetaminophen is due to toxic metabolites N-acetyl-p-benzoquinoneamine (NABQI), which is produced by cytochrome P-450 enzymes (Neuman, 2019). Normally this metabolite is detoxified by conjugating with glutathione, however, during overdose, the metabolite produced in high concentration which overwhelmed the detoxification process and lead to hepatocellular injury and cell death.

In hepatotoxicity reactive oxygen species (ROS) are produced which destroy the cell membranes integrity and releasing the cytosolic enzymes like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) into the bloodstream and elevating thiobarbituric acid reactive substances (TBARS) level (AL-Janabi, Khadairi *et al*., 2020). Hepatocellular damage is also associated with alterations in other biomarkers like, glutathione (GSH), superoxide dismutase (SOD) and Malondialdehyde (MDA), also change the hematological indices like, Packed cell volume, Hb, total leucocyte count (TLC), MCH, MCHC and MCV (Islam *et al*., 2019) Thus ROS is linked with cell apoptosis, causes oxidative stress and damage to biomolecules like lipids, nucleic acids, proteins, and carbohydrates. Natural products (herbal origin) have antioxidant constituents like phenolic compounds, flavonoids, triterpenoids, tannin and alkaloids that can prevent the oxidative damage by normalizing the effect of free radicals (Abdullahi and Hamza, 2020).

**Corresponding author:* e-mail: basheer.icup@gmail.com

In the present study, *Typha elephantina* (family Typhaceae), a bush like small plant which was locally known as Hogla, was selected to assess its therapeutic value.(Singh, Narwal *et al*., 2020) The plant is native to North Africa, India, Nepal, Pakistan and Iran and grows in the Sundarban woodland as well as in other areas (Raj *et al*., 2018). The plant being used traditionally as a natural remedy e.g. The leaves are diuretic and the pollen is astringent, desiccant, diuretic, haemostatic, vulnerary (Rao *et al*., 2016). The mentioned plant have various pharmacological findings such as anti-inflammatory, anti-pyretic, anti-tumor, anthelmintic (Musara and Aladejana, 2020). As part of our medicinal flora of Pakistan, the mentioned plant leaves aqueous extract was studied for the hepatoprotective, other serum and biochemical activities.

MATERIALS AND METHODS

Sample collection and extraction

The fresh leaves of *Thypa elepentenia* (T.E) were collected from District Swat and were identified at the Department of Botany, University of Malakand, Khyber Pakhtoonkwa, Pakistan for further process.

The fresh leaves of (T.E) (1430gram) were chopped and grinded with a mechanical grinder. The ground material was then extracted three times with deionized water (2500 mL) to prepare the aqueous extract (640mL). The filtrate was eliminated from the plant material by a muslin cloth and cleaned from all kind of debris through filter paper. Finally the filtrate was changed to crude extract (382.3 gram) red black color past using rotary evaporator machine (Japan made) Buchi R-210) and was then stored at 4°C in the refrigerator to avoid the fungal attack (Alirezalu *et al*., 2020). The whole experiment was carried in the biochemistry laboratory at the department of biotechnology, University of Malakand Khyber Pakhtoonkwa Pakistan.

Treatment regime

The experimental animals (male rabbits) were divided into seven groups, five animals in each, according to the "Guide for the Care and Use of Laboratory Animals" (Council, 2010) (Extract and Chemicals were given orally to all animals for 21 days continually. The treatment regime is given below.

1) Group N: Normal control without any treatment.
2) Group T: Induced toxicity with paracetamol (PCM) only at dose rate 300mg/kg body weight (BW)
3) Group A: Received *Typha elephantina* leaves aqueous extract (TE.AQ) at dose 100mg/kg BW and PCM (300 mg/kg BW)
4) Group B: Administered with (TE.AQ) at dose rate 200 mg / kg BW and PCM (300mg/kg BW)
5) Group C: Treated with 300mg/kg BW, (TE.AQ) and PCM at dose 300mg/kg BW
6) Group D: Feed with (TE.AQ) only at a dose 200mg/ kg BW
7) Group E: Administered with silymarine 50mg/kg BW and PCM at dose 300mg/kg BW

Blood collection for hematological and serum biochemical analysis

Blood was collected from the rabbits of all groups by carotid bleeding into centrifuge tubes at different days i.e. on day 7th, week first (W1), day 14th, week second (W2) and finally on day 21st, week third (W3). The blood samples were centrifuged for 10mints at 3000 rev/hr. in a bench centrifuge to obtain clear serum, which was used for the assessment of hematological and biochemical analysis (Ahmed and Sheikh, 2019).

Histopathological analysis

At the end of experiment all animals were dissected and livers were excised from corresponding group animals and immediately stored in a solution having 10% formalin and 0.9% NaCl. The tissues were then embedded in paraffin, thinly sectioned using a microtome, stained with haematoxylin and eosin (H&E) for conventional morphological assessment, then observed under light microscope (BX50; Olympus, Tokyo). The images were achieved by a digital camera system (Pixcera Co., Osaka, Japan) attached to the microscope (Sobhani and Roomiani *et al*., 2019).

Ethical approval

Study was conducted as per approval protocols (notification no: FAB14/2020-64), in accordance with the animal's byelaws 2008, Scientific Procedures.

STATISTICAL ANALYSIS

Results of the present study were analyzed by applying, one-way ANOVA and Tukey test, using graph pad prism, demo version 5 (www.grap pad.com), at significance $P < 0.05$ respectively.

RESULTS

Blood hematological analysis

The consumption of (PCM) significantly ($P<0.05$) decreased the blood HB, RBC count, HCT value, MCV value, MCH and MCHC levels, while an increased was noticed in WBC, PLT count, lymphocytes, neutrophils and monocytes concentration. The ingestion of extract (TE.AQ) on day 21st (W3) of the experiment at doses of extract 200 mg (group B) to 300mg/kg (group C) BW significantly ($P<0.05$) normalized the hematological factors but extract at dose rate 100 mg (group A) caused non-significant effect even at day 21st of treatment. However no significant change was observed during the first and second week (W1, W2) of treatment, with all doses of extracts as shown in (tables 1 and 2).

Serum biochemical analysis

A statistically significantly (P<0.05) elevation was observed in the AST level, ALT and ALP values, when the animals were treated with PCM (300mg/kg.BW). The (TE.AQ) extract showed tonic effects on the levels of serum biochemical markers. The effect was non-significant in the first week (W1) of treatment with all doses of (TE.AQ) extracts. However a significant (P<0.05) normalized effect was observed during the second and third week (W3 and W3) of experiment when compared to control and silymarine groups. It was found that an increase in the dose (300 mg/kg BW) has strong remedial effects on serum biochemistry as shown in (table 3). Highly significant (p≤0.05) increases in serum cholesterol, triglycerides, LDL and significant decrease in HDL were observed in paracetamol-treated rabbits (group T), but on day 21st (W3) of experiment, a high statistically significant (p≤0.05) decreases in serum cholesterol, triglycerides and LDL and increase in HDL was observed with administration of (TE.AQ) extract at dose 300mg/kg BW (group C), when compared to rabbits in the control group (table 4). Meanwhile silymarine feed rabbits (group E) showed decease in serum cholesterol, triglycerides and HDL while increase in LDL levels on day 21, (W3) of experiment. While in the first two weeks (day 7th and 14th), extract treatment at all doses i.e. 100mg/kg BW, 200mg/kg BW and 300mg/kg BW, showed no significant effects on serum cholesterol, triglycerides, HDL and LDL when compared to both normal control group and toxic control group. In other hand only extract feed animals (group D) has maintain normal serum lipid profile during three weeks of experiment.

GSH levels in liver

Paracetamol (PCM) induced hepatic and renal toxicity, as significant (p<0.05) reduction was observed in liver glutathione (GSH) of rabbits in groups-T when compared with group-N. The animals of group-A, showed similar results as that in group-T animals, while group-B, animals showed slightly recovery but not significant. This indicates that the low dose extract (100mg/kg BW, has no curative effect on GSH levels of liver tissues, while the (TE.AQ) extract at dose rate of 200mg/kg body weight has a mild effect but not significant. In another hand group-C, animals were treated with high dose of (TE.AQ) extract (300mg/kg BW) significantly (p<0.05) improved the GSH levels of liver when compared with group-N and group-T respectively. The results of animals feed with high dose of (TE.AQ) extract were comparable with group-E, which has ingest the silymarine at dose of 200 mg/kg BW, significantly raise the GSH levels. The animals of group-D, were administered with extract only (200mg/kg body weight, without PCM intoxication prior or after the treatment, has sustained the improve GSH level of liver as shown in (table 5).

Radical scavenging activity RSA level of liver tissue

The effects of the increasing dosages of (TE.AQ) extract (100, 200 and 300mg/kg BW) produced dose dependent, significant (p<0.05) increase in the reduced %RSA levels of paracetamol hepatotoxic rabbits after treatment when compared with those of the paracetamol and normal control rabbits (group- N and group- T) respectively. % RSA levels were significantly lower in paracetamol control groups when compared to all other treatment groups. (TE.AQ) extract at 100mg/kg body weigh administration to group A, animals, has no effect on reduced % RSA levels. A slight but not significant(p < 0.05) increase was observed in % RSA level in rabbits of group B, after the treatment of (TE.AQ) extract at 200 mg/kg body weight. However a statistically significant (p <0.05) increase in % RSA level was observed in animals of group C with ingestion of 300mg/kg body weight of (TE.AQ) extract when compared with PCM and normal control animals. Silymarine at dose of 100mg/kg BW significantly increase the % RSA level, when feed to group E, rabbits after intoxication with PCM. The rabbits of group D, has sustain, the improve level of % RSA, as they were administered with 200mg/kg body weight, of (TE.AQ) extract only having no PCM intoxication prior or after treatment shown in (table 5) respectively.

TBRAS level in liver tissue

The level of the Thiobarbituric acid reactive substances (TBARS) is a marker of lipid peroxidation. Animals exposed to PCM (300g/kg BW) showed a significant rise (p<0.05) in TBARS level in the liver compared to control (group N vs. group T,). Animals (group A) exposed to PCM, followed by (TE.AQ) leave extract at dose 100 mg/g body weight, showed no decreases in TBARS level, producing similar results like group T. (TE.AQ) extract at 200 m/kg BW, non-significantly reduced the TBARS level in group B animals, when compared to normal control animals. In group-C a significant (P<0.05) decrease in TBARS level in the liver was observed with 300mg/kg body weight (TE.AQ) extract administration when compared to normal control and toxic control group (group N and group T). Experimental group-E, feed with silymarine at 100mg/k body weight, showed significant (p<0.05) decrease in TBRAS level after intoxication with PCM. Meanwhile a significantly stable TBRAS level was maintained in animals of group D, ingest only (TE.AQ) extract, having no PCM toxicity, when compared with group-N (control animals) shown in (table 5).

The antioxidant activity of aqueous extract of T. elephantina on the basis of percent inhibition of DPPH free radical

The results for antioxidant activity against DPPH are shown in (table 3. 6). The percent inhibition values were: 45.66%, 48.96, 53.71%, 72.70% and 76.55%. At lowest concentration (20ppm) percent inhibition was 45.66%, followed by 60ppm, 80ppm, 100ppm and 200ppm respectively. The increased in extract concentration

Table 1: Mean and standard deviation (M ± SD) of blood erythrocytes and other related hematological factors of different experimental animal groups, treated at different doses, at different days.

Groups	RBC x10^3/µL			HB g/dL			MCV g/dL			MCH g/dL			MCHC g/dL		
	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
N	6.3± .59	6.6 ±0.55	7.0± 0.67	12± 0.76	13± 0.78	13± 1.4	71± 3.6	68± 3.3	71± 1.9	32± 3.1	32± 2.8	33± 2.6	33± 2.4	33± 2.6	34± 1.1
T	2.1± 0.30+	2.6± 0.55+	3.1± 0.74+	8.5 ±0.5 7+	8.2 ±0.82 +	8.1± 0.19 +	34± 2.1+	35± 2.6+	35± 2.6+	26± 0.84 +	22± 1.0+	17± 1.0+	20 ±0.96 b+	21± 1.1+	19± 1.1 +
A	2.5 ± 0.27+	3.2± 0.45+	4.6± 0.28+	8.4± 0.86 +	8.3 ±0.71 +	8.9± 0.35 +	37± 3.1+	41± 1.3+	47± 3.1+	19± 0.55 +	19± 0.55 +	21± 0.83 +	22 ± 1.5+	23± 0.55+	22± 0.8 3+
B	2.7± 0.44+	3.6± 0.58+	4.9± 0.18*	9.2 ±0.9 3+	9.3 ±0.86 +	10± 0.62 *	38± 2.7+	48± 0.89*	58 ±1.5*	19± 1.1+	22± 0.84*	21± 1.1*	22 ± 1.1+	23 ±0.84 +	27± 1.1 *
C	3.6± 0.34*	5.1 ±0.5*	6.2± 0.5	10± 0.58 *	10± 0.93*	12± 1.1	46± 1.8*	51± 2.1*	67± 2.7	24± 1.2*	25± 0.84*	30± 1.7	25± 1.6*	27± 1.3*	32± 1.7
D	6.5± 0.51*	6.8± 0.84*	7.0± 0.69	12 ±1.2 *	11 ±0.8	11± 0.9	67± 3.7*	65± 4.7*	71± 1.6	31± 0.6	31± 0.5	32± 1.6	31 ±0.9*	31 ±0.6*	33 ±2. 7
E	5.3± .084*	5.3 ±0.08 4*	6.9± 0.57*	9.4± 1.1	9.7± 0.65*	11± 0.82	50 ±0.89 *	62 ±2.7*	70± 1.5	23 ±2.9 *	29± 2.0*	31± 1.5	27± 1.7*	32 ±0.1*	33± 0 .5

Table 2: Mean and standard deviation (M ± SD) of blood leucocytes and other related hematological factors of different experimental animal groups, treated at different doses and at different days.

Groups	WBC x10^3/µL			PLT g/dL			Neutrophils g/dL			Lymphocytes %			Monocytes %		
	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
N	6.7 ±0.49	7.8 ±1.1	7.9± 0.8	145 ±7.5	144± 0.3	144 ±3	37± 2.3	36 ±2.4	38± 2.3	32± 1.9a	33± 2.6	34 ±1.7	32± 1.9	12 ±1.6	14 ±1.7
T	15± 2.8+	16± 1.5+	16± 1.5+	266 ±2.2 +	271± 4.1+	272± 0.8	62± 3.2+	67± 1.7+	69± 1.6+	72± 2.9+	69± 1.1+	70± 0.77 +	72± 2.9b	24± 1.5+	27± 0.96+
A	15± 0.7+	14 ±0.5+	14 ± 0.4+	258± 1.5+	245 ±1.3+	238 ±3.2+	57 ±3.1+	51± 3.2+	48± 5.5*	64± 3.6+	64± 0.3+	60± 2.3+	64± 3.6+	23 ±0.5+	21± 0.82*
B	13± 0.55+	13± 0.44+	12 ± 0.74*	244± 5.6+	221± 2.6+	204± 2.6*	49 ±1.6*	45 ± 2.3*	44 ± 2.7*	65± 3.7+	59±1 .1	41± 1.9*	65± 3.7+	20 ±0.5+	18± 1.7*
C	11± 0.79*	11 0.71d	7.2 0.86	201 ± 2.5*	181 ±4.5*	150± 1.3	45 ± 1.8*	43± 1.8*	39 ±1.7	48± 0.89*	42± 1.9*	36± 1.2	48± 0.8*	17± 1.7*	14± 1.4
D	7.0 ± 1.6	8.4 ±1.0	8.4± 1.0a	149± 6.2	143± 3.1	143 ±3.6	36 ± 1.9	37± 1.9	37 ±1.5	34± 0.71	35± 2.3	37 ±2	34± 0.71	12± 0.96	13± 0.82
E	10± 0.45c	8.4 ±0.54*	7.4± 0.54	182± 4.1*	175± 4.6*	151± 4.5	47± 1.5*	39± 2.2*	38 ±2.8	45± 2.2*	40± 1.8*	38± 1.7	45± 2.2d	16 ±1.4*	16± 1.5

Note: += significant difference from control animals (group N) while * = significant difference from toxic control animals (group T) at (P<0.05.

caused an increase in percent inhibition. These increases in percent inhibition represent the antioxidant potential of (TE.AQ) extract.

Histopathological al analysis

Animals of group =N, showed normal liver parenchyma showed that endothelia linings of central veins had normal morphology and no evidence was found for pericentral fibrosis shown in fig. 1(A1). Kupffer cells showed non-reactivity. No Pathological symptoms were found.

Group=T received paracetamol (PCM) at dose rate 300 mg/kg BW), Toxic liver; liver parenchyma shows sever inflammation, necrosis and bile duct proliferation. Hepatocyte shows ballooming and orientation of hepatic cord was distorted with degeneration shown in (fig. A2).

Group = A feed with extract (100 mg/kg BW) and (PCM 300 mg/kg BW showed inflammation and peri cellular fibrosis. Inflammation, necrosis, swelling of hepatocytes, degeneration is seen, shown in (fig. A3). Animals of group =B, that received extract 200 mg/kg BW and PCM 300mg/kg BW), displayed liver distortion with scattered granulomas along hepatocyte hepatocytes, degeneration is seen (fig. A4). Extract (300mg/kg BW) and PCM 300 mg/kg BW), group= C, Liver parenchyma reveals mostly normal. Mild type inflammation is seen. No necrosis, no degeneration, no swelling hepatocytes are seen shown in (fig. A5).

Liver of group = D, feed with extract only reveals mostly normal parenchyma. No inflammation, necrosis and no degeneration are seen, hepatic portal vain and artery

Table 3: Mean and standard deviation (M ± SD) of serum biochemical parameters of different experimental animal groups, treated at different doses and at different days.

Variables	Serum ALT			Serum AST			Serum ALP		
Groups	W1	W2	W3	W1	W2	W3	W1	W2	W3
N	41±1.6	39±4.3	38±4.9	46±3.2	45±4.3	41±1.3	47±2.0	44±3.0	46± 3.8
T	180± 2.8+	186±5.4+	187±2.1+	182± 6.6+	186±2.7+	185±4.7+	176±1.7+	182±4.4+	186±7.7+
A	163± 2.7+	132±6.8*	124±3.4*	160± 5.7+	128±2.1*	119±3.6c*	169±2.4+	122±2.8*	90±5.1*
B	165± 4.4+	120 ±7.2*	96 ±3.2*	98±12+	85±3.7*	64±6.3*	116±6.3+	86±3.1*	84±4.8*
C	133± 3.6*	62 ±3.4*	44± 3.3	83± 3.9*	77±4.0*	46±2.3	94±4.0*	64±1.5*	54±2.8
D	47 ±4.4	47 ±3.2	34 ±1.2	47± 1.5	44 ± 0 .7	41±1.5	53±3.8	47±2.7	52±2.6
E	80± 3.2*	61 ±3.2*	44± 4.5	76± 3.8*	58± 2.9*	39±3.2	83 ±3.2*	66±2.6*	55±30

Table 4: Mean and standard deviation (M ± SD) of serum lipid profile of different experimental animal groups, treated at different doses and different days.

Groups	CHOL			HDL			LDL			T.G		
	W1	W2	W3	W1	W2	W3	W1	W2	W3	W1	W2	W3
N	54± 3.7	52± 4.5	48± 4.5	45± 3.3	44± 1.2	43± 2.8	23± 2	25± 2.1	25± 1.7	54± 2.8	53± 4.3	48± 5.0
T	85± 4.3+	88± 9.1+	91±9.1 +	25 ±2.3+	24± 1.2+	23± 1.4+	60± 2.3 +	65± 2.2+	69± 1+	143± 6.2+	150± 5.5+	159± 3.6+
A	81± 3.9+	79± 3.5+	73± 3.5+	29± 1.8+	30± 1.6*	30± 1.8*	56± 3.6+	51± 1.9+	46± 3.4*	98± 2.8+	86± 4.6*	72± 4.4*
B	78± 2.5+	75± 3.5+	65± 3.5*	30± 0.3+	31± 2.8*	35 ±2.5*	52 ±4b	43 ±3.6*	42± 2.4*	89± 2.3c	77± 1.8*	65± 1.5*
C	71± 1.7*	60± 3.2*	52± 2.2	33± .2+	36± 1.6*	39± 1.3	42± 2.2*	34 ±2.3*	28 ±1.6	72± 2.7*	67± 1.5*	56± 2.6
D	55± 3.2	52± 3.6	48± 3.6	40± 2.5	42 ±1.7	43 ±2.2	26± 2.2	23± 2.3	24± 1.8	59± 2.1	56± 2.6	50± 2.4
E	69± 1.4*	61± 6.9*	49± 4.9	39± 2.8*	38± 1.5*	39± 2.2	35± 3.6*	35± 4.1*	29 ±1.5	73± 3.3*	58± 4.1*	51± 3.6

Table 5: GSH, RSA and TBARS of liver tissues in different experimental animal groups, treated with different doses of extracts and chemicals

Groups	GSH Liver	RSA liver	TBARS liver
N	39.25±0.71	58.69±1.084a	12±1.7
T	14.06±1.18+	27.73 ±1.179b	34±1.4+
A	19.31±0.74+	38.13±0.7489c	32±0.82+
B	27.81±1.88*	41.41±1.271d	17±1.5
C	39.76±0.72	57.56±1.366a	13±0.66
D	40±0.57	57.56±1.366a	11.9±2.2
E	39.34±0.82	56.34±1.432a	10.3±1.74

Note: += significant difference from control animals (group N) while * = significant difference from toxic control animals (group T) at (P<0.05.

showed normal structure (fig. A6). Group = E animals were administered with silymarine (100mg/kg BW) and PCM 300mg/kg BW), Normal liver parenchyma and hepatocytes seen normal with background of inflammation. No necrosis, no degeneration, no swelling hepatocytes are seen (fig. A7)

DISCUSSION

Typha elephantina have anti-inflammatory and wound healing activities (Rahman, Chakrabarty *et al*., 2014). However the plant has not been well explored. Since this study was arranged to evaluate therapeutic value of aqueous extract of *Typha elephantina* (T.E) leaves in paracetamol intoxicated male rabbits.

Over dose of paracetamol (PCM) ingestion can injured the liver of the experimental animals, as a results reactive oxygen species (ROS), are produced which destroy the cell membranes integrity causing cell death and exerting oxidative stress (Wang *et al*., 2017). Present study showed that ingestion of (PCM) disturbed the blood hematology, liver enzymes like ALT, AST and ALP and serum biomarkers such as creatinine, urea and uric acid along with lipid profile also alter the histomorphological architecture. The (TE.AQ), extract was found to have hepatoprotective potential by reducing the alanine transaminase (ALT), aspartate transaminase (AST) and alanine transaminase (ALP) and other serum biomarkers like creatinine, urea ad uric acid levels towards normal range. Different doses of (TE.AQ extract i.e (100mg/kg

Table 6: The antioxidant activity of Crude extract on the basis of percent inhibition of stable, DPPH free radical

Concentration	No	Absorption	% Inhibition
50 ppm	1 2 3	0.50 0.100 0.85	45.66 %
100 ppm	1 2 3	0.178 0.190 0.150	48.96 %
150 ppm	1 2 3	0.171 0.128 0.169	53.71 %
200 ppm	1 2 3	0.130 0.117 0.129	72.70 %
300 ppm	1 2 3	0.052 0.083 0.103	76.55 %

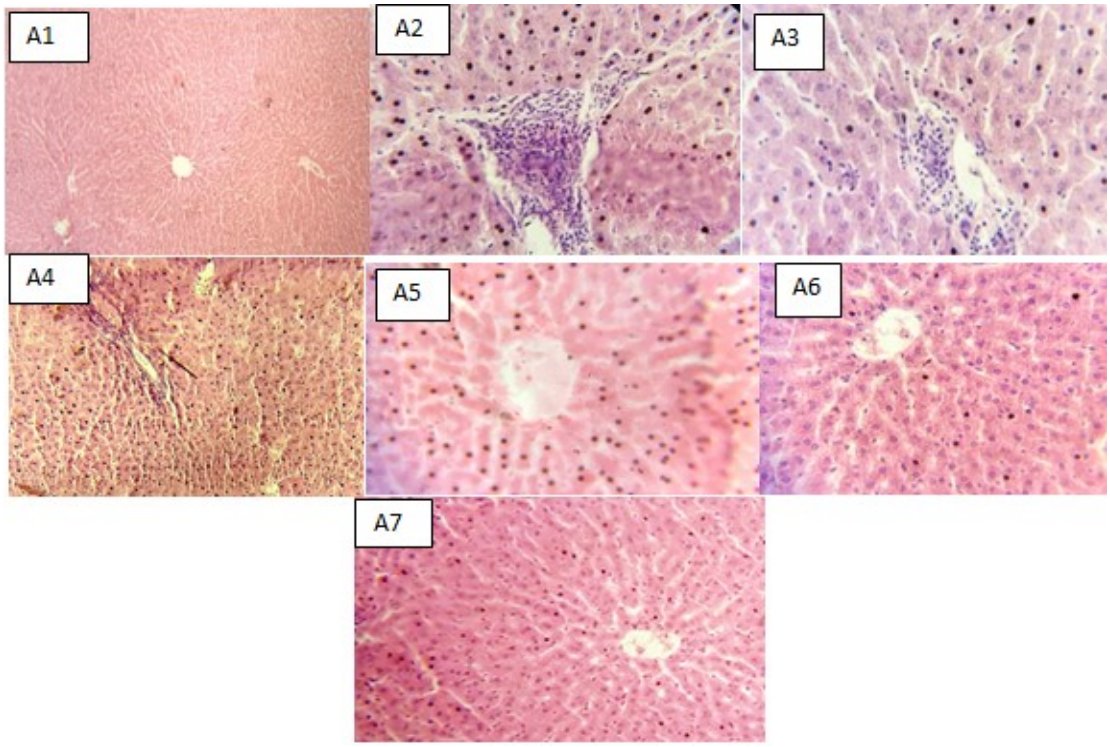

Fig. 1: Liver histopathological representation of normal control animals and all other experimental animals groups i.e (A1, A2, A3, A4, A5, A6 and A7).

body weight 200mg/body weight) during the first and second weeks of treatment showed no significant effect. However at the end of third week, the high dose (300mg/kg body weight) of (TE.AQ) extract significantly reduced the serum enzymes. Since the animals that received only extract without PCM ingestion, were found to maintained, improved serum biochemical and histopathological status from start till the end of research work. These remedial effects of (TE.AQ) extract are due to the antioxidant activity and lysosomal membrane stabilization. These effects were comparable to silymarine treated group, a stander hepatoprotective agent (Salem, Shaban *et al*., 2018). Similar study was also conducted by (Safaei i *et al*., 2018) who studied the protective effect of *Parthenium Hysterophorus* against CCL4 and paracetamol induced toxicity.

Serum lipids are related with the onset of several heart ailments. The cholesterol (CH), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) are more common in evaluating heart problems (Sultani *et al*., 2020): (D'Annunzio, Donato *et al*., 2012).

In the present study, it was noticed that liver damage due to PCM overdose is linked with significant rise in serum glucose and lipid levels i.e., cholesterol, TG, LDL and reduced in HDL. Treatment with (TE.AQ) extract at dose rate 300 mg/kg body weight has remarkably decreases serum glucose and lipid levels such as cholesterol, LDL, triglyceride level and increase HDL level. High concentration of HDL- cholesterol is considered as good cholesterol, related to prevent cardiac complications as it is liable in removing excess cholesterol by carrying it to the liver (Chiesa and Charakida, 2019). Glucose is crucial to lipid digestion. It forms pyruvic acid through glycolysis or to be changed into fatty acids, (Bechmann *et al*., 2012). The effect of PCM intake is also prominent in the case of hematology of animals, that significant ($p<0.05$) reduced the red blood cells (RBC), hemoglobine (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobine (MCH) and mean corpuscular hemoglobine concentration (MCHC) values and increased the white blood cells (WBC), platelets (PLT), lymphocyte, neutrophil and monocyte levels. Similar study has also been established, that demonstrate, hepatotoxicity can change haematological indices, such as packed cell volume, Hb total leucocyte count (TLC), MCH, MCHC and MCV suggesting interference with haematological profile (Oladele, Oyeleke *et al*., 2020). Analyzing of hematological indices can be used to determine the range of toxic effect of any exogenous substance including plant extract on the blood composition of an animal (Arika, Nyamai *et al*., 2016). High dose of (TE.AQ) extract significantly increase the lower levels of RBC, HB, and MCV, MCH and MCHC and reduced WBC, PLT, lymphocyte, neutrophil and monocyte concentration toward normal range. This normalization of the hematological indices was found in the third week of treatment. The remedial effect was significant at the high dose of (TE.AQ) extract (300mg/kg body weight), while the lower and moderate dose (100mg/kg and 200 mg/ kg body weight) extract showed no significant effects. The significant increase in the hematological parameters with *Thypa elephantina* (TE.AQ) extract, suggest that it may contain phytochemicals that may activate erythropoietin production in the stem cells of experimental animals. Erythropoietin is a glycoprotein hormone which motivates stem cells in the bone marrow to revenue red blood cells (Fibach, 2019). The present study is in agreement with (Oshilonya, IJioma *et al*., 2015) who reported the effects of *Caulis bambusae* (Bamboo) stem extract on hematological and biochemical parameters in chinchilla rabbits (Sun, Yu *et al*., 2013).

Tissues damage triggered by oxidative stress can be controlled by exogenous antioxidants and endogenous antioxidants defense system of the host, the endogenous defense consist of enzymatic and non-enzymatic antioxidants, like as superoxide dismutase SOD, catalases CAT and glutathione GSH (He, He *et al*., 2017). GSH is powerful nucleophilic, 3-peptide (L-γ-glutamyl cysteinyl glycine), antioxidant, important for cellular defense, that detoxifies, reactive oxygen species via binding with and eliminating of toxic molecules, thus handling the inflammatory cytokine chain reactions (Pes, 2013). Reduced levels of GSH in various tissue makes the defense system weak, against reactive oxygen species, and may lead to per-oxidative damage. In present study, it is concluded that, paracetamol administration reduced the GSH levels in liver by the process of lipid peroxidation, may have resulted free radicals generation. The levels of GSH and %RSA in the liver was significantly lowered in paracetamol hepatotoxic rabbits compared to the control rabbits indicate impaired antioxidative defense and injury of liver.

Administration of TE.AQ extracts at different doses; to the paracetamol treated rabbits. High dose extract 300 mg/kg body weight has raised the levels of GSH and % RSA in the liver highlighting the antioxidant activity of the plant extracts are dose dependent. The detected lower level of GSH in paracetamol toxic rabbits might be due to high levels of reactive substances scavenging activity, that were generated as a result of the necrosis and apoptosis of liver and renal cells or possible decline in hepatic and renal production of GSH (Zeb and Ullah, 2015). Results of the present study are analogous to the findings of (Khan, 2017), who investigated the Protective effect of *Aerva jevanica* against ethanol Induced hepatic stress in rats, he concluded that *Aerva jevanica* (AJME) possess antioxidant activity and hepatoprotective effects. Silymarine also improved the antioxidant enzymes level and concentration of glutathione was boosted, while reduction in lipid peroxidation were detected dose dependently with silymarin and extract (Afsar, Razak *et al*., 2020).

High level of ROS destroys cellular lipids, proteins and nucleic acids. To avoid this situation antioxidant defenses have developed to eradicate most of these oxidant mediators. Imbalance between oxidative impairment and defensive mechanisms caused oxidative stress (Chan and Ho, 2015). (Sabiu,*et al*., 2015), has presented that the high levels of lipid peroxides enhanced level of TBARS in the liver and of PCM ingested rabbits. Our results showed that in PCM control animals the levels of TBARS was high in liver, due to increased lipid peroxidation. Animals treated with high dose of (TE.AQ) extract, the TBARS levels were low in liver. This decreased in TBARS level may be due to active ingredients of (TE.AQ) extract that may responsible for scavenging ROS and improving the antioxidant potentials of tissues.

CONCLUSION

From the present study it was concluded that the *Typha elephantina* leave's aqueous extract (TE.AQ) is very potent against liver damage. . The extract is also beneficial for the normalization of hematological and serum lipid parameters. In addition, (TE.AQ) extract has potent antioxidant capacity revealed form good healing ability by improving and regulating the impaired levels of serum glutathione GSH, radial scavenging assay (RSA) and thiobarbituric acid reactive substances (TBARS). The precise mechanism of action of the plant extracts is not known in lowering lipid peroxidation but their antioxidant properties may have played a major role. The most essential constituents of plant are their Phenolic compounds, alkaloid and carotenoids. These compounds are very potent against acute or chronic liver disorders including, cancer and heart diseases because of their anti-oxidant potential (Alok, Jain *et al*., 2014).

Therefore one can use this plant (TE.AQ) extract traditionally, for many liver disorders. Further this plant extract can also be used scientifically for other fatal and chronic diseases. Hence this research recommends that the (TE.AQ) extract possess the anti-oxidant property, which could be used as best source of advance medication. Thus further exploration of the plant is required

REFERENCES

Abdullahi R and Hamza A (2020). Physiological roles of phenolic compounds isolated from medicinal plants of tropical origin. *Int. J. Glob. Sustain.,* **6**(4): 133-142.

Afsar T, Razak S, Almajwal A and Al-Disi D (2020). Doxorubicin-induced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; Improvement by Acacia hydaspica tannin-rich ethyl acetate fraction. *Saudi J. Biol. Sci.,* **27**(9): 2251-2260.

Ahmed I and Sheikh ZA (2019). Hematological and serum biochemical parameters of five freshwater snow trout fish species from river Jhelum of Kashmir Himalaya, India. *Comp. Clin. Path*., **28**(3): 771-782.

AL-Janabi AIH, Khadairi MM, Al-amari MJ. Hirallah AAK (2020). Curative role of vitamin (C) in reduction of cadmium toxicity on the levels of some liver functions, lipid peroxidation and antioxidants enzymes in *in vivo* condition. *Plant Arch.,* **20**(2): 936-940.

Alirezalu K, Pateiro M, Yaghoubi M, Alirezalu A, Peighambardoust SH and Lorenzo JM (2020). Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive review. *Trends Food Sci. Technol.,* 292-306.

Alok S, Jain SK, Verma A, Kumar M, Mahor A and Sabharwal M (2014). Herbal antioxidant in clinical practice: A review. *Asian Pac. J. Trop. Biomed.,* **4**(1): 78-84.

Arika W, Nyamai D, Musila M, Ngugi M and Njagi E (2016). Hematological markers of *in vivo* toxicity. *J. Hematol. Thrombo. Dis.,* **4**(2): 1000236.

Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M and Canbay A (2012). The interaction of hepatic lipid and glucose metabolism in liver diseases. *J. Hepatol.,* **56**(4): 952-964.

Chan KWK and Ho WS (2015). Anti-oxidative and hepatoprotective effects of lithospermic acid against carbon tetrachloride-induced liver oxidative damage *in vitro* and *in vivo*. *Oncol. Rep.,* **34**(2): 673-680.

Chiesa ST and Charakida M (2019). High-density lipoprotein function and dysfunction in health and disease. *Cardiovas. Drugs Therapy,* **33**(2): 207-219.

Council NR (2010). Guide Care Laboratory Animals, National Academies Press.

D'Annunzio V, Donato M, Buchholz B, Pérez V, Miksztowicz V, Berg G and Gelpi RJ (2012). High cholesterol diet effects on ischemia-reperfusion injury of the heart. *Can. J. Physiol. Pharmacol.,* **90**(9): 1185-1196.

Fibach E (2019). Erythropoiesis *In vitro* a research and therapeutic tool in Thalassemia. *J. Clin. Med.,* **8**(12): 2124.

He L, He T, Farrar S, Ji L, Liu T and Ma X (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell. Physio. Biochem.,* **44**(2): 532-553.

Islam MO, Bacchetti T and Ferretti G (2019). Alterations of antioxidant enzymes and biomarkers of nitro-oxidative stress in tissues of bladder cancer. *Oxid. Med. Cell. Longev.,*1-10., 10.1155/2019/2730896

Khan RA (2017). Protective Effect of *Aerva jevanica* against ethanol induced hepatic stress in rats: A randomized control report. *Ind J. Pharm. Edu. Res,* **51**(2): S110-S114.

Kirman C, Li A, Sheehan P, Bus J, Lewis R and Hays S (2020). Ethylene oxide review: Characterization of total exposure via endogenous and exogenous pathways and their implications to risk assessment and risk management. *J. Tox. Envir. Health, Part B.,* pp.1-29.

McCrae J, Morrison E, MacIntyre I, Dear J and Webb D (2018). Long-term adverse effects of paracetamol a review. *Br. J. Clin. Pharmacol.*. **84**(10): 2218-2230.

Musara C and Aladejana EB (2020). Typha capensis (Rohrb.) NE Br.(Typhaceae): Morphology, medicinal uses, biological and chemical properties. *Plant Sci. Today,* **7**(4): 578-583.

Neuman MG (2019). Biomarkers of drug-induced liver toxicity. *Ther. Drug Monit.,* **41**(2): 227-234.

Oladele JO, Oyeleke OM, Awosanya OO, Olowookere BD and Oladele OT (2020). Fluted Pumpkin (Telfairia occidentalis) protects against phenyl hydrazine-induced anaemia and associated toxicities in rats. *Adv. Trad Med,* pp.1-7.

Oshilonya H, IJioma S and Ibeh I (2015). Prevalence of type-2 diabetes mellitus amongst suspected subjects in Agbor, Delta State, Nigeria and its relationship with age and gender. *Arch. Appl. Sci. Res.,* **7**(3): 18-20.

Patel Rajesh M. Patel Natvar J (2011). *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *J. Adv. Pharm. Edu. Res.,* 152-168.

Pes L (2013). Study of the non-proteinogenic delta-amino acid ACCA, its biological investigation and application. Doctoral Thesis, University College Dublin. School of Chemistry and Chemical Biology, Dublin, Ireland.

Rahman MM, Chakrabarty JK, Muhit MA. Dash PR (2014). Evaluation of analgesic activity of the different fractions of *Typha elephantina* Roxb. *Int. J. Pharmacog.,* **1**(6): 380-383.

Raj AJ, Biswakarma S, Pala NA, Shukla G, Kumar M, Chakravarty S and Bussmann RW (2018). Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal, India. *J. Ethnobiol. Ethnomed.,* **14**(1): 8.

Rao MRK, Saranya Y, Divya D and Linn A (2016). Preliminary phytochemical analysis of Typha domingensis rhizome aqueous extracts. *Int. J. Pharm. Sci. Rev. Res.,* **37**(1): 30-32.

Sabiu S, Sunmonu TO, Ajani EO and Ajiboye TO (2015). Combined administration of silymarin and vitamin C stalls acetaminophen-mediated hepatic oxidative insults in Wistar rats. *Revista Brasileira de Farmacognosia.,* **25**(1): 29-34.

Safaei F, Mehrzadi S, Khadem Haghighian H, Hosseinzadeh A, Nesari A, Dolatshahi M, Esmaeilizadeh M and Goudarzi M (2018). Protective effects of gallic acid against methotrexate-induced toxicity in rats. *Acta Chirurgica Belgica.,* **118**(3): 152-160.

Salem GA, Shaban A, Diab HA, Elsaghayer WA, Mjedib MD, Hnesh AM and Sahu RP (2018). Phoenix dactylifera protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats. *Biomed. Pharmacother*., **104**(1): 366-374.

Singh G, Narwal S and Agnihotri S (2020). *Typha elephantina* Roxb.: A review on ethanomedicinal, morphological, phytochemical and pharmacological perspectives. *Res. J. Pharm. Tech.,* **13**(11): 5546-5550.

Sobhani B, Roomiani S, Ahmadi Z and Ashrafizadeh M (2019). Histopathological analysis of testis: Effects of astaxanthin treatment against nicotine toxicity. *Iran J. Toxicol.,* **13**(1): 41-44.

Sultani R, Tong DC, Peverelle M, Lee YS, Baradi A and Wilson AM (2020). Elevated triglycerides to high-density lipoprotein cholesterol (TG/HDL-C) ratio predicts long-term mortality in high-risk patients. *Heart Lung Circulation,* **29**(3): 414-421.

Sun J, Yu J, Zhang P-C, Tang F, Yue YD, Yang YN, Feng ZM and Guo XF (2013). Isolation and identification of lignans from Caulis Bambusae in Taenia with antioxidant properties. *J. Agric. Food Chem.,* **61**(19): 4556-4562.

Wang X, Wu Q, Liu A, Anadón A, Rodríguez JL, Martínez-Larrañaga MR, Yuan Z and Martínez MA (2017). Paracetamol: Overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds *in vivo* and *in vitro*. *Drug Metabol. Rev.,* **49**(4): 395-437.

Weaver RJ, Blomme EA, Chadwick AE, Copple IM, Gerets HH, Goldring CE, Guillouzo A, Hewitt PG, Ingelman-Sundberg M and Jensen KG (2020). Managing the challenge of drug-induced liver injury: A roadmap for the development and deployment of preclinical predictive models. *Nat. Rev. Drug Discov.,* **19**(2): 131-148.

Zeb A and Ullah S (2015). Sea buckthorn seed oil protects against the oxidative stress produced by thermally oxidized lipids. *Food Chem.* **186** (1): 6-12.