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Evaluation of hepatoprotective action of *Solanum melongena* l. peel extract against paracetamol induced liver damage in albino rats

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ABSTRACT

Introduction: Paracetamol or also called as acetaminophen or APAP is known from long time to induce hepatotoxicity in human and as well as experimental animals. Free radical injury and oxidative stress can be accounted for pathogenesis and progression of hepatic toxicity. In this context out of many naturally reported anti-oxidants, *Solanum melongena* is known by many common names; to name a few as egg plant or brinjal was selected due to its high anti-oxidant potential, easy palatability and also potential to act as functional food. From ethnobotanical claims, it's reported to cure degenerative ailments through its strong anti-oxidant potential. *Materials and methods*: Extraction of *S. melongena* L. purple fruit peels were conducted in water: ethanolic mixture (1:1) and aqueous media to get SMHA and SMAQ, respectively. Following OECD test guideline 423, acute oral toxicity was conducted in Swiss Webster mice with SMHA and SMAQ at75, 150, 250, 500, 1000, and 2000 mg/kg/bw. There were no signs of evident toxicity in mice. So, 100, 200, and 400 mg/kg of SMHA and SMAQ was selected and orally administered to PCM induced hepatotoxic Wistar rats. Serum bio-chemical estimation (fasting sugar, AST, ALT, ALP, TC, TG, and HDL) was conducted after 24 hours post PCM treatment. Tissues were subjected to bio-chemical tests for estimating liversuperoxide dismutase (SOD), glutathione reductase (GSH) level. Microscopical examination was performed on liver cells. *Results*: In rats, serum and liver biomarker enzymes, LDL levels and elevated glucose levels were attenuated in dose dependent manner and were in normal ranges post treatment with SMHA and SMAQ when compared with Silymarin group rats. From histopathological observations, it was established that there was reduced fatty deposits or adipocytes infiltration in hepatocytes. This is a potential marker for recovery of hepatocytes against hepatotoxicity by PCM following lipid peroxidation mechanism. Statically all the findings concerning serum and tissue pathology was at $p<0.05$. *Conclusion*: The present study established that *S. melongena* L. possess excellent anti-oxidant potential and thereby subsequent hepato-protective activity too by attenuating free radicals and elevated liver biomarker enzymes. From functional food point of view, it can be used as both preventative as well as curative agent in liver compromised people. It evinced future potential for a new drug development for treating liver impairment.

Keywords: fruit peel, functional food, hepatoprotective, Nasunin, *Solanum melongena* L.

1. INTRODUCTION

Acetaminophen (APAP) or Paracetamol (PCM) was discovered in Germany at the end of 19th century. Today PCM or chemically known as N-acetyl-p-aminophenol tops the list for being the most commonly used over-the-counter antipyretic and analgesic drugs (Rocha et al., 2005). Therapeutically it is reported as safe at recommended therapeutic dose. Its potential hepatotoxic side effect was not uncovered till clinical reports started coming following over dose as reported by Davidson and Eastham 1966. However worldwide concerns are raining as its have been identified to cause acute liver failure (ALF, Larsen *et al.*, 2014). From safety profile point of view it's an excellent drug of choice however due to over dose doses it causes hepatotoxicity. Main target is liver for PCM. It causes primary lesion in acute centrilobular hepatic necrosis. At 150-250 mg/kg it has potential to cause severe liver damage as this is the reported as single acute threshold dose but it varies as there is inter individual susceptibility (Hemabarathy *et al*., 2009).

PCM post metabolism in liver by cytochrome P-450 system generates n-acetyl p-benzoquinonimine (NAPQ1). This metabolite generation is the main cause of hepatotoxicity as this reactive metabolite bind covalently to macromolecules and causes cell damage (Vermeulen et al., 1992) causing glutathione depletion that increases liver susceptibility to oxidative stress (Cohen and Khairallah 1997). In this way an oxidative stress is created on liver that affects various antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) (Banu et al., 2012; Habib et al., 2015; Faras et al., 2017). These complications can be avoided if antidotes are administered early. However due to rise in scientific researches on herbal medicine and newer concept of functional foods, a naturally presenting antidote is already available through dietary consumption that can show protective action against PCM. As per World Health Organisation, 80% of the world uses remedies based on plant as primary healthcare system (Hemabarath *et al*., 2009).

From time and ages, various poly-herbal formulations have been used and prescribe by health practitioners as they have been store houses of natural antioxidants which act synergistically together to protect the liver cells. However with passage of time and modernisation, due to restricted availability of these ingredients, high cost, degraded or adulterated quality of ingredients, and difficult palatability; it has raised a silent question over the capability of these to help recover liver from oxidative stresses. So, there is pressing need for a low cost, easy palatability and ease of availability of hepatoprotective agents that can cover and cater across all socio-economic strata which can act as both prophylactic and reparative remedy. Therefore after exhaustive literature search, it was found that *Solanum melongena* L. tops the chart with few more herbal agents that have very high anti-oxidant activity due to presence of an anthocyanin component Nasunin. Bluish purple colour can be attributed to Nasunin, which derives its name form

nasu; means blue colour in dyeing industry in Japan. Also it has been a food of choice in South Asian cuisines both from health and nutritive point of view serving the public as functional food.

So, to prove the ethnobotanical claim, present *in-vivo* pharmacology study was systematically designedwith the extract of peels of *S. melongena* purple coloured fruit for its hepato-protective activity using contemporary technologies and well established experimental model. Already an *in-vitro* study has been reported with peels that established very high anti-oxidant activity (Sarkar et al., 2019). So, in context to this, a first of its kind, an *in-vivo* hepatoprotective study was designed with purple coloured peels to scientifically prove that the anti-oxidant potential of Nasunin in the brinjal peel is the major contributor among many anti-oxidants for brinjal's strong anti-oxidant activity. Both aqueous (AQ) and hydro-alcohol extract (HA) of *S. melongena* (SM) purple coloured fruit peels (SMAQ and SMHA) were evaluated for *in-vitro* anti-oxidant activity.

2. MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were purchased from registered suppliers in India. Paracetamol (PCM) was procured from Sigma-Aldrich, Milwaukee, USA; Silymarin as from Silybon Suspension; carboxymethyl cellulose (CMC) from Himedia; Ethanol, Reduced glutathione (GSH), Sulphuric acid, Tri-chloroacetic acid (TCA), and 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB) from Molychem, India; Anaesthetic ether, Formalin from Thomas Baker (Chemicals), and Liver enzymes (AST, ALT, ALP and Bilirubin), Total cholesterol (TC), Triglycerides (TG), and High Density Lipo-polysaccharide (HDL) from Reckon Diagnostics Pvt. Ltd.

Extraction from peels

Solanum melongena purple peeled fruits were purchased from local market in Gurgaon, Haryana, India and authenticated by plant taxonomist Dr. K. Madhava Chetty, Sri Venketeswara University, Tirupati, Andhara Pradesh, India and a voucher specimen number of 1894 has been retained in Sri Venketeswara University. From purple fruits, green coloured crowns were removed. Peels were removed in very thin layer from purple egg plant fruits peels were shade dried. Dried peels were coarsely powdered and subjected to the Soxhlet extraction procedure using hydro-alcohol (50%; v/v). Additionally, they were also subjected to maceration at 50 ^{o}C to obtain aqueous extract. Both the extracts were filtered, distilled off and concentrated in a rotary evaporator under reduced pressure 40 ^{o}C. These crude extracts were used for further study (Sarkar et al., 2019).

Ethical committee approval number

The experimental animal protocol was approved by the Institutional Animal's Ethics Committee with protocol no. KRMU/CPCSEA/RES/IAEC-2018(1) and was in accordance with guidelines of the regulatory body of the government.

Animal husbandry conditions

Animals were kept as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Swiss Webster mice (male) of weight range 20-25 g and Wistar rats (adult male) of weight range 200-225 gm were housed in standard environmental conditions in polypropylene cages (seven per cages) maintained at 23 ± 2˚C with a 12 h light-dark cycle. During entire experimental phase including 1 week as an acclimatisation, mice and rats were fed with standard laboratory rodent diet and normal tap water for drinking purpose in inverted steel sipper fitted polypropylene bottles supported on the top of the cage; *ad libitum* respectively.

Induction of hepato-toxicity

Body weight of animals

Before oral PCM administration, each animal was weighed and animals with similar weight were grouped. Body weight of each group was measured periodically till the end of the study.

Animal experiment

SMHA and SMAQ extracts of purple peels of *S. melongena* were subjected to acute oral toxicity study to determine further doses levels for Wistar rat hepatoprotective models.

Acute oral toxicity study

Swiss mice (3/group) were fasted for 4 hours pre-dosing (Kandimalla *et al.*, 2016). Then animals were orally dosed with SMHA and SMAQ extract of *S. Melongena* at 75, 150, 250, 500, 1000, and 2000 mg/kg body weight (BW) following OECD test guideline 423

(Mishra *et al.,* 2013). These doses were administered orally to mice with the exception of the control group and observed for morbidity and mortality after day 1, day 7 and day 14 (Aliyu *et al.*, 2015).

PCM induced hepatotoxicity

Based on oral acute toxicity studies, 100, 200, and 400 mg/kg of SMHA and SMAQ extracts were selected for oral administration, respectively (Rodriguez *et al.*, 2016). Male Wistar rats were divided into 9 groups (6/group). Group 1 was normal untreated or negative control group, that were fed with standard diet and distilled water all the time without any treatment dose; Group 2: Hepatotoxic positive control (H) were rats were treated with PCM orally at a single dose of 3.0 g/kg in 0.2 % CMC; Group 3: H + 100 mg/kg SMHA; Group 4: H + 200 mg/kg SMHA, Group 5: H + 400 mg/kg SMHA, Group 6: H + 100 mg/kg SMAQ, Group 7: H + 200 mg/kg SMAQ, Group 8: H + 400 mg/kg, and Group 9: H + 150 mg/kg silymarin (standard hepatoprotective drug) which was considered as standard treatment group. After 24 hours of PCM administration, blood was collected and biochemical analysis was carried out to estimate serum glucose, lipid profile (total cholesterol TC, triglyceride TG, HDL levels), liver enzymes and bilirubin (Saraswat *et al.*, 1993).

Post 24 hours of PCM induction, treatment of animals with peel extracts and standard was continued for the next 14 days. After 24 hours of the last treatment, all the animals were weighed, sacrificed, blood collected while liver was removed, weighed and stored for histopathological studies.

Serum biochemical estimation of animals

Under ether light anaesthesia from retro-orbital route, blood was collected. Biochemical parameters such as blood glucose, lipid profile and liver function tests were estimated on 0^{th}, 7^{th} and 14^{th} day of PCM induction. Blood was centrifuged at 4000 rpm for 15 minutes at 4˚C, and serum was separated. Biochemical estimation was carried out using commercially available kits of Reckon Diagnostics Pvt. Ltd.

Serum biochemical estimations

Biochemical estimation like fasting blood glucose, AST, ALT, ALP, bilirubin, TG, TC, and HDL level were done with the help of diagnostic kits.

Tissue biochemical assays for estimating antioxidant levels

In liver homogenate level of antioxidant enzymes *i.e.*, glutathione reductase (GSH) and superoxide dismutase (SOD) was estimated:
Preparation of tissue homogenate: Liver from experimental groups were harvested; rinsed with ice-cold saline and homogenized in 0.1 M Tris-HCl buffer, pH 7.4 at 4°C. Homogenates were subjected to centrifugation at 3,000 rpm for 10 minutes at 4°C using refrigerated centrifuge. Supernatant was collected, and used for the biochemical estimations. The protein concentration in the tissue homogenate was measured by BSA method (Kandimalla *et al.*, 2016).

Estimation of reduced glutathione

The reduced glutathione (GSH) was estimated according to the method described by Beutler *et al.*, 1963. Equal volume of the supernatant of tissue homogenate was mixed with 10% w/v TCA. Mixed well; centrifuged (1000 rpm; 4˚C for 10 minutes) and supernatant was collected. Supernatant was added with 2 ml of 0.3 M disodium hydrogen phosphate and 0.25 ml of 0.001 M DTNB. Absorbance was measured at 412 nm & results expressed as µM protein.

Estimation of superoxide dismutase

To 50 µl of homogenate was mixed with 1.15 ml of distilled water followed by 1.2 ml of sodium pyrophosphate buffer of pH 8.3 (0.052 M), 300 µl of NBT (300 µM) and 200 µl of NADPH (780 µM). Potassium phosphate buffer 50 µl of pH 7.5 (0.1 M) was added for control reading replacing enzyme source. Absorbance was measured in both control and test samples to calculate the SOD enzyme activity. The activity was expressed as unit enzyme/mg protein.

Histopathology

Post euthanasia, liver was harvested. Liver was collected in 10% neutral buffered formalin solution followed by dehydration in ethanol and embedded in paraffin. Sections of 5µm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H & E) dye for microscopic observations.

Statistical analysis

Statistical analysis was carried out by GraphPad Prism 7 software, and one-way analysis of variance (ANOVA) followed by Tukey's multiple tests was used for statistical analysis. Values were expressed as mean ± SEM. Following symbols (*#) were assigned for comparison of different groups: Normal control *vs* positive control (*); Hepatotoxic control *vs* 100, 200, 400 mg/kg of plant extracts and standard drug (#); $p<0.05$ was considered significant.

3. RESULTS

Acute toxicity studies

There was no adverse effect or mortality observed in mice at dose ranges of up to 2000 mg/kg of SMHA and SMAQ extracts of *S. melongena* peels. All animals were alive, healthy and active during the observation period of 14 days. Therefore, SMHA and SMAQ extracts of *S. melongena* were considered safe and non-toxic up to 2000 mg/kg and the LD_{50} was considered more than 2000 mg/kg. On the basis of acute toxicity study, different doses *i.e.* 100, 200 and 400 mg/kg of SMHA and SMAQ were selected for the *in-vivo* study.

PCM-induced hepatotoxicity

The morphological and biochemical parameters including body weight (BW), fasting serum glucose level, liver function test (serum AST, ALT, ALP and bilirubin) as well as lipid profile (serum TC, TG, and HDL) were assessed.

Effect of SMHA and SMAQ extracts of *S. melongena* on BW

BW was measured at the beginning of the study (0 or basal day), and 14th day of the study. On 0 day, there was no significant difference found in body weight of normal control rats (185.87 ± 2.45) and hepatotoxic control rats (186.55 ± 2.91 g) on basal day. However, on 14th day, hepatotoxic control rats were observed with a loss in body weight to 169.27 ± 2.67 g in contrast to normal control rats (210.67 ± 2.76 g).

Oral intervention with different doses (100, 200 & 400 mg/kg) of SMHA improved body weight from 184.65 ± 2.09 g to 192.50 ± 1.82 g (100 mg/kg), 187.67 ± 2.05 g to 194.38 ± 2.10 g (200 mg/kg) and 185.20 ± 1.50 g to 197.29 ± 2.43 g (400 mg/kg) in comparison to hepatotoxic control group (186.55 ± 2.91 g to 169.27 ± 2.67 g). Similarly, SMAQ also exhibited an improvement in body weight to 198.45 ± 2.40, 201.0 ± 2.41, 206.74 ± 2.42 g at 100, 200 & 400 mg/kg doses, respectively. Silymarin at 150 mg/kg also showed improved body weight of experimental rats i.e., 204.58 ± 2.89 g (Figure 1).

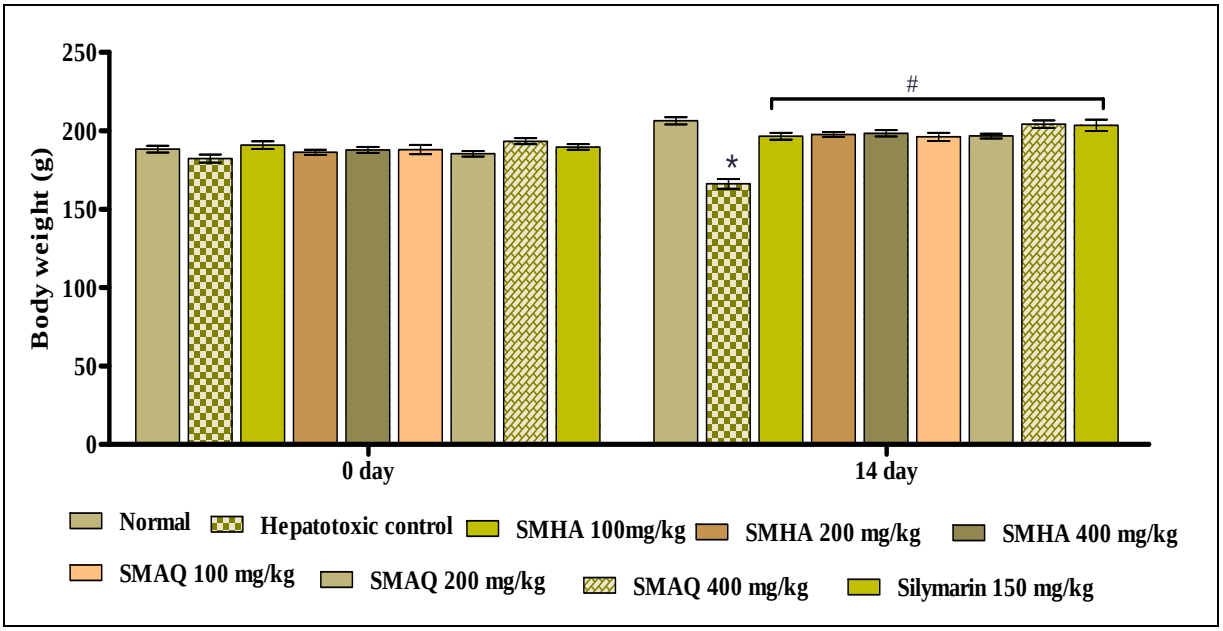

Figure 1 Effect of SMHA and SMAQ extracts of *S. melongena* on body weight (g) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of *S. melongena* on serum glucose level

The fasting glucose level of hepatotoxic control rats was found to be significantly increased in comparison to the normal control rats ($p<0.05$) at 14th day. Oral administration of SMHA and SMAQ at different doses exhibited a significant dose-dependent reduction of

elevated glucose level at 14th day. SMHA at 100, 200 & 400 mg/kg doses showed significant reduction of serum glucose level in comparison to hepatotoxic control rats ($p<0.05$). Similarly, different doses of SMAQ (100, 200 & 400 mg/kg) showed marked reduction in glucose level ($p<0.05$), respectively in comparison to hepatotoxic control rats. Silymarin at 150 mg/kg dose showed almost similar reduction level of glucose level (Table 1).

Table 1 Effect of SMHA and SMAQ extracts of *S. melongena* on fasting glucose level (mg/dL) in PCM- induced hepatotoxic rats

Groups	Day 14
Normal control	99.42 ± 1.63 mg/dL
Hepatotoxic control	146.38 ± 2.76 mg/dL
SMHA 100 mg/kg	126.45 ± 2.24 mg/ dL
SMHA 200 mg/kg	120.56 ± 1.00 mg/dL
SMHA 400 mg/kg	118.37 ± 1.39 mg/dL
SMAQ 100 mg/kg	124.29 ± 1.12 mg/ dL
SMAQ 200 mg/kg	121.29 ± 1.82 mg/dL
SMAQ 400 mg/kg	116.3 ± 1.67 mg/dL
Silymarin 150 mg/kg	117.7 ± 1.31 mg/dL

Effect of SMHA and SMAQ extracts of *S. melongena* on liver function

Effect of SMHA and SMAQ extracts of S. melongena on serum Aspartate Aminotransferase (AST) level

A significant elevation in AST level was observed in hepatotoxic control rats (78.39 ± 2.01 mg/dL) in comparison to normal control group (48.72 ± 1.89 mg/dL) *($p<0.05$)*. Oral administration of 100, 200 & 400 mg/kg doses of SMHA significantly reduced the elevated level of serum AST in hepatotoxic rats i.e. 60.8 ± 1.38, 55.28 ± 1.55 and 51.32 ± 1.61 mg/dL, respectively in comparison to hepatotoxic control rats at the end of study. Similarly, SMAQ (100, 200 & 400 mg/kg) reduced the AST level to 56.90 ± 2.10, 52.21 ± 1.29 and 49.19 ± 1.31 mg/dL, respectively ($p<0.05$) in comparison to hepatotoxic control group. Silymarin at 150 mg/kg dose showed a marked reduction in AST level to 50.12 ± 1.03 mg/dL. The results of SMHA and SMAQ were found to be comparable to the Silymarin (Figure 2).

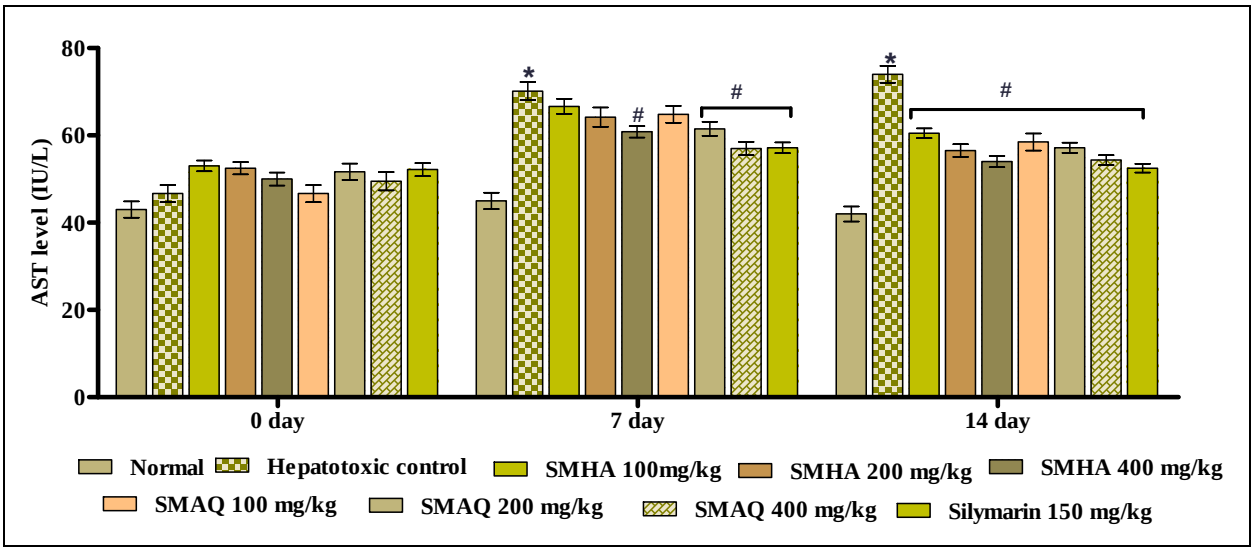

Figure 2 Effect of SMHA and SMAQ extracts of *S. melongena* on serum AST level (mg/dL) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of S. melongena on serum Alanine Aminotransferase (ALT) level

ALT level was found to be elevated in hepatotoxic control rats (73.57 ± 1.721 mg/dL) while compared to normal control group (50.29 ± 0.78 mg/dL) ($p<0.05$). Oral administration of 100, 200 & 400 mg/kg doses of SMHA significantly reduced the elevated level of serum ALT level in hepatotoxic rats. i.e. 54.45 ± 2.01, 51.28 ± 1.01 and 49.23 ± 0.99 mg/dL, respectively in comparison to hepatotoxic control rats at the end of study. Similarly, SMAQ (100, 200 & 400 mg/kg) reduced the ALT to 51.09 ± 0.69, 49.25 ± 1.29 and 47.21 ± 1.32 mg/dL, respectively ($p<0.05$) in comparison to hepatotoxic control group. Silymarin at 150 mg/kg dose showed a

marked reduction in ALT level to 46.19 ± 1.41 mg/dL. The results of SMHA and SMAQ were found to be comparable to the standard drug (Figure 3).

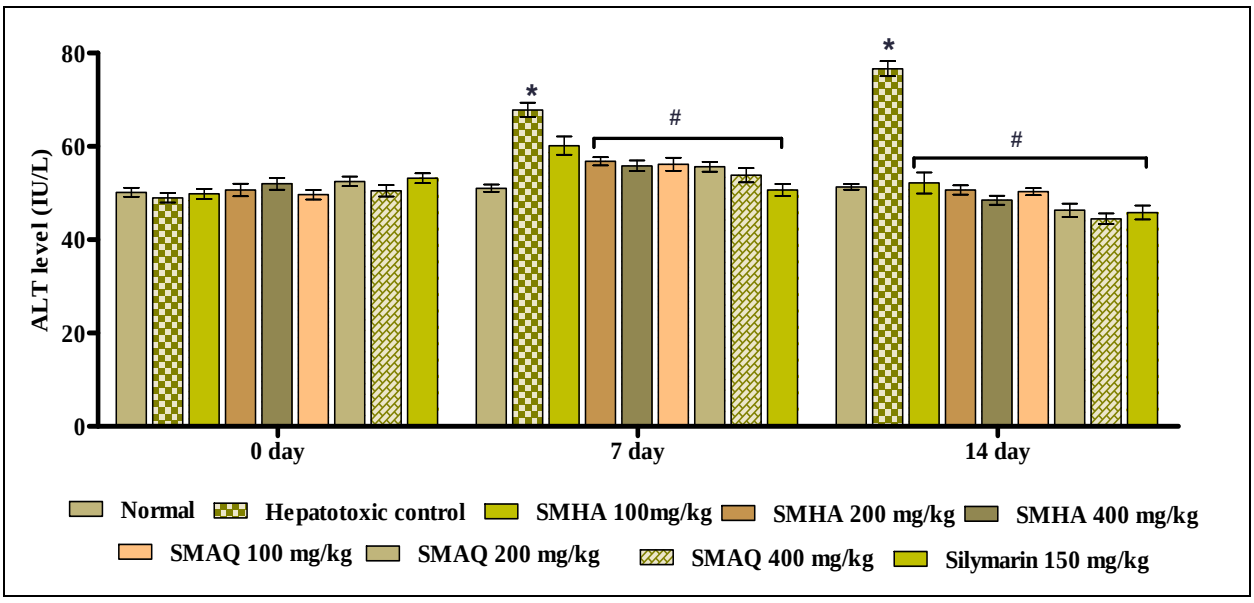

Figure 3 Effect of SMHA and SMAQ extracts of *S. melongena* on serum ALT level (mg/dL) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of S. melongena on serum Alkaline Phosphatase (ALP) level

ALP level was found to be elevated in hepatotoxic control rats (331.54 ± 2.90 mg/dL) while compared to normal control group (183.38 ± 2.97 mg/dL) ($p<0.05$). Oral administration of 100, 200 & 400 mg/kg doses of SMHA significantly reduced the elevated level of serum ALP level in hepatotoxic rats i.e. 232.56 ± 2.38, 228.29 ± 3.01 and 216.23 ± 2.99 mg/dL, respectively in comparison to hepatotoxic control rats at the end of study. Similarly, SMAQ (100, 200 & 400 mg/kg) reduced the ALP to 224.19 ± 2.96, 218.45 ± 4.01 and 210.38 ± 3.89 mg/dL, respectively ($p<0.05$) in comparison to hepatotoxic control group. Silymarin at 150 mg/kg dose showed a marked reduction in ALP level to 214.98 ± 3.65 mg/dL. The results of SMHA and SMAQ were found to be comparable to the standard drug (Figure 4).

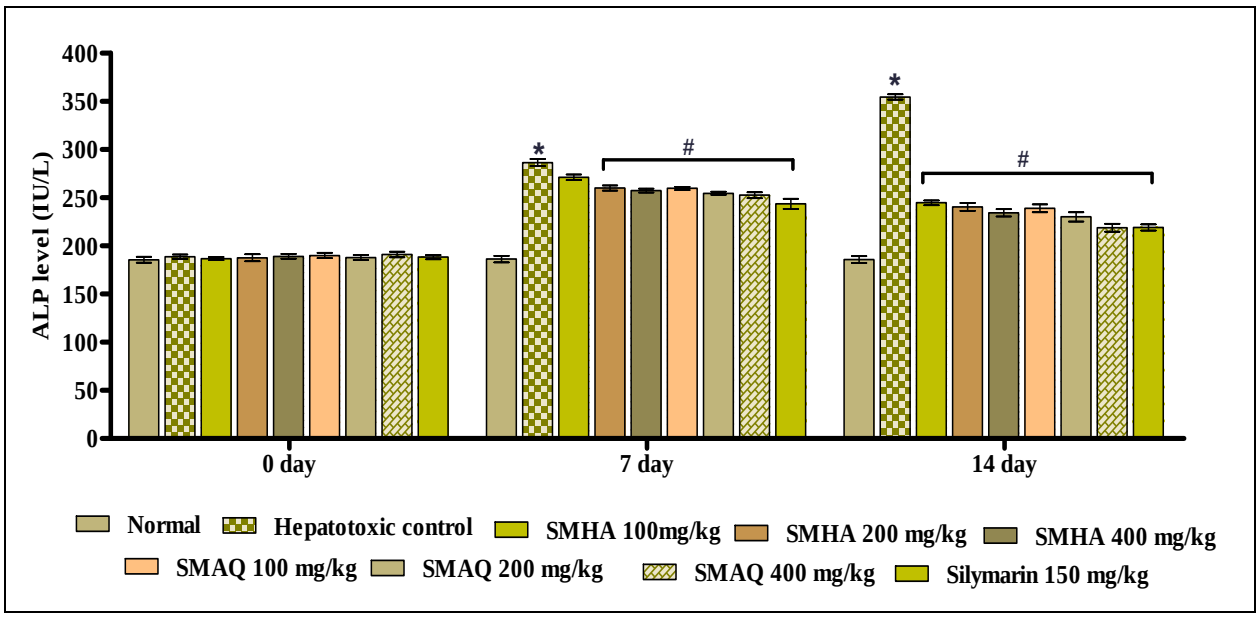

Figure 4 Effect of SMHA and SMAQ extracts of *S. melongena* on serum ALP level (mg/dL) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of S. melongena on serum total bilirubin level

Bilirubin level was found to be elevated in hepatotoxic control rats (1.10 ± 0.023 mg/dL) while compared to normal control group (0.25 ± 0.014 mg/dL) ($p<0.05$). Oral administration of 100, 200 & 400 mg/kg doses of SMHA significantly reduced the elevated level of serum total bilirubin level in hepatotoxic rats i.e. 0.49 ± 0.013, 0.44 ± 0.014 and 0.41 ± 0.019 mg/dL, respectively in comparison to hepatotoxic control rats at the end of study. Similarly, SMAQ (100, 200 & 400 mg/kg) reduced the total bilirubin to 0.46 ± 0.01, 0.42 ± 0.012 and 0.38 ± 0.012 mg/dL, respectively ($p<0.05$) in comparison to hepatotoxic control group. Silymarin at 150 mg/kg dose showed reduction in bilirubin level to 0.37 ± 0.013 mg/dL (Figure 5).

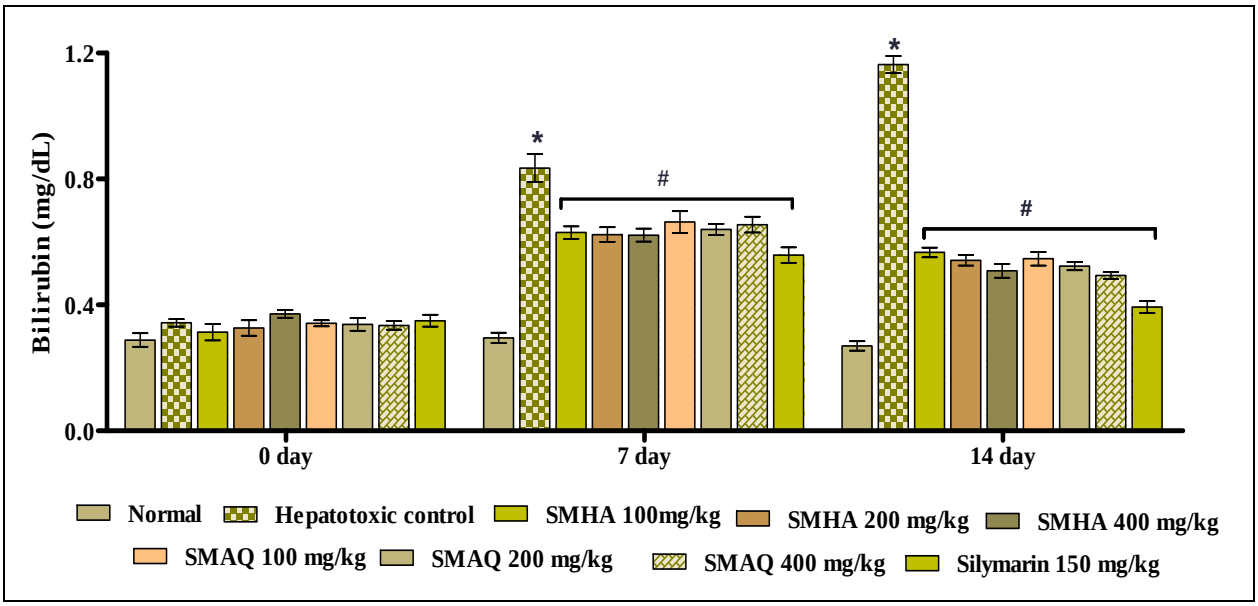

Figure 5 Effect of SMHA and SMAQ extracts of *S. melongena* on serum total bilirubin level (mg/dL) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of *S. melongena* on lipid profile

Effect of SMHA and SMAQ extracts of S. melongena on serum triglyceride level

Serum level of triglycerides augmented in hepatotoxic control rats in comparison to normal control rats ($p<0.05$). A significant reduction in serum triglycerides level was observed in hepatotoxic rats treated with SMHA and SMAQ (100, 200 & 400 mg/kg doses) respectively in comparison to hepatotoxic control rats. Silymarin at 150 mg/kg dose showed reduction in triglycerides level to ($p<0.05$) (Table 2).

Effect of SMHA and SMAQ extracts of *S. melongena* on serum total cholesterol level

A significant augmentation in total cholesterol level was observed in hepatotoxic control rats in comparison to normal control group ($p<0.05$). Oral administration of 100, 200 & 400 mg/kg doses of SMHA sand SMAQ significantly reduced the elevated level of serum total cholesterol in hepatotoxic rats, respectively in comparison to hepatotoxic control rats at the end of study. Silymarin at 150 mg/kg dose showed reduction in bilirubin level too (Table 2).

Effect of SMHA and SMAQ extracts of S. melongena on serum high-density lipoproteins (HDL) level

Serum level of HDL was significantly reduced in hepatotoxic control group in comparison to normal control group ($p<0.05$). Administration of SMHA and SMAQ extract at doses of 100, 200 & 400 mg/kg doses resulted in increase in serum HDL. Silymarin also significantly improved the level of serum HDL (Table 2).

Table 2 Effect of SMHA and SMAQ extracts of *S. melongena* on serum triglyceride, total cholesterol and HDL level (mg/dL) in PCM-induced hepatotoxic rats

Parameter / Groups	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)
Normal control	78.83 ± 4.21	80.19 ± 2.39	37.98 ± 0.78
Hepatotoxic control	120.50 ± 3.46	117.28 ± 2.87	23.29 ± 0.97

SMHA 100 mg/kg	86.66 ± 3.46	89.38 ± 2.26	29.00 ± 0.57
SMHA 200 mg/kg	84.83 ± 2.85	85.29 ± 2.18	31.02 ± 0.79
SMHA 400 mg/kg	83.26 ± 1.56	84.15 ± 2.39	33.18 ± 0.63
SMAQ 100 mg/kg	87.08 ± 0.93	86.38 ± 2.10	34.28 ± 0.57
SMAQ 200 mg/kg	85.16 ± 0.87	85.29 ± 2.03	36.67 ± 0.78
SMAQ 400 mg/kg	80.50 ± 2.08	83.15 ± 2.22	37.45 ± 0.57
Silymarin 150 mg/kg	76.20 ± 2.35	75.19 ± 2.28	36.02 ± 0.57

Effect of SMHA and SMAQ extracts of S. melongena on liver weight

Hepatotoxic control rats were observed with a considerable increase in weight of liver (8.16 ± 0.05 g) when compared to normal control group (6.76 ± 0.069 g) ($p<0.05$). Oral intervention of 400 mg/kg doses of SMHA significantly reduced the weight of liver in hepatotoxic rats to 7.64 ± 0.08 g in comparison to hepatotoxic control rats at the end of study. However, SMHA 100 and 200 mg/kg doses did not produce any significant changes. On the other hand, SMAQ (100, 200 & 400 mg/kg) reduced the liver weight to 7.80 ± 0.07, 7.59 ± 0.07 and 7.46 ± 0.05 g, respectively. Silymarin at 150 mg/kg dose also showed reduced weight of liver (7.55 ± 0.06 g) (Figure 6).

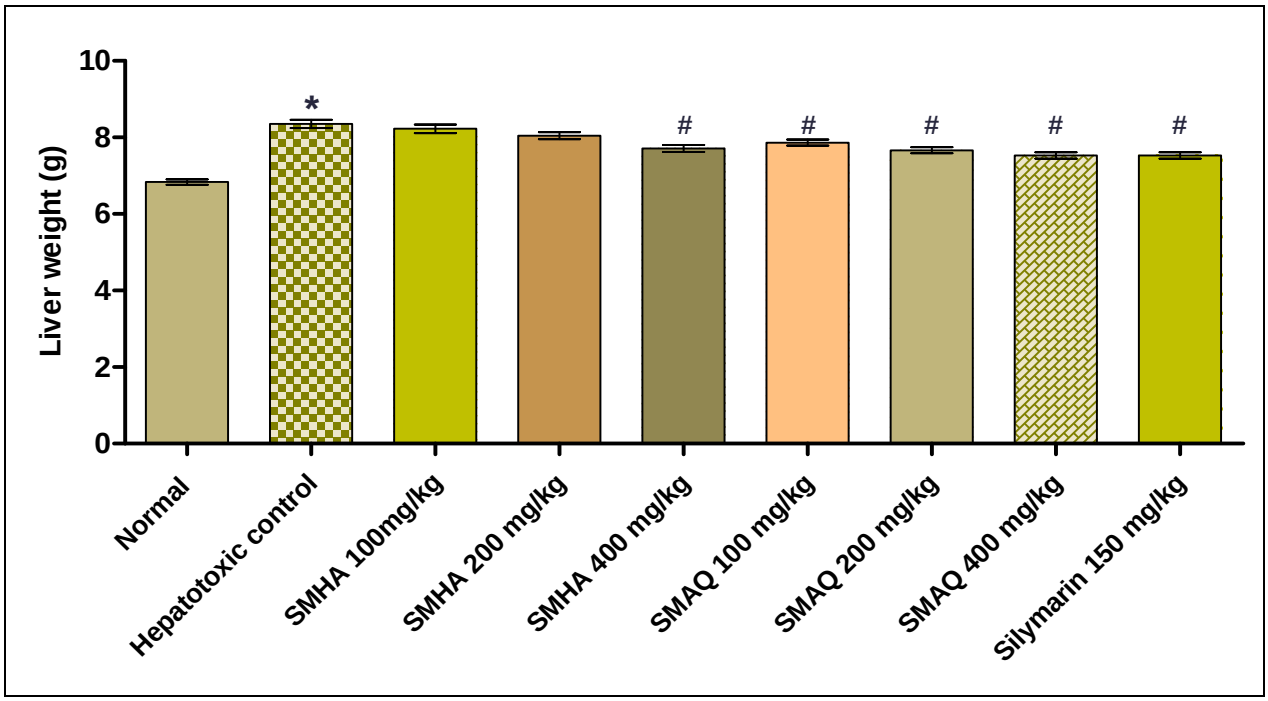

Figure 6 Effect of SMHA and SMAQ extracts of *S. melongena* on liver weight (g) in PCM- induced hepatotoxic rats

Effect of SMHA and SMAQ extracts of *S. melongena*on antioxidant enzymes

Effect of SMHA and SMAQ extracts of S. melongenaon glutathione reductase (GSH) level in liver

The level of GSH (Beutler *et al.,* 1963) in liver was significantly reduced in hepatotoxic control group in comparison to normal control group ($p<0.05$). Oral administration of SMHA and SMAQ (100, 200 & 400 mg/kg) for 14 days elevated the level of GSH in a dose dependent manner, respectively in comparison to hepatotoxic control group. SMHA (100, 200 mg/kg) and SMAQ (100 mg/kg) did not produce significant changes. Silymarin at 150 mg/kg dose augmented GSH level (Table 3).

Effect of SMHA and SMAQ extracts of S. melongena on superoxide dismutase (SOD) level in liver

The level of SOD in liver was significantly reduced in hepatotoxic control group in comparison to normal control group ($p<0.05$). SMHA at 200 & 400 mg/kg doses significantly elevated the level of SOD in a dose dependent manner in comparison to hepatotoxic control group. However, SMHA at 100 mg/kg did not produce any significant difference in SOD enzyme level. Moreover, the level of SOD in rats receiving SMAQ (100, 200 & 400 mg/kg) was also found to be improved. Silymarin at 150 mg/kg dose also improved SOD level ($p<0.05$) (Table 3).

Effect of SMHA and SMAQ extracts of S. melongena on lipid peroxidation (Thiobarbituric acid reactive species; TBARS)

Lipid peroxidation was measured in terms of TBARS (Okhawa *et al.*, 1979) which is an index of lipid peroxidation in hepatotoxic rats that signals increased levels of oxygen free radicals. The level of TBARS was found to be significantly elevated in hepatotoxic control rats ($p<0.05$). SMHA (100, 200 & 400 mg/kg) reduced the level of TBARS in a dose dependent manner in comparison to hepatotoxic control group. The level of TBARS in rats receiving SMAQ (100, 200 & 400 mg/kg) was also reduced. Silymarin at 150 mg/kg dose also significantly reduced TBARS level (Table 3).

Table 3 Effect of SMHA and SMAQ extracts of *S. melongena* on GSH, SOD and TBARS level in PCM- induced hepatotoxic rats

Parameter Groups	GSH (mM/mg protein)	SOD (U/mg protein)	TBARS (nmol/mg protein)
Normal control	60.29 ± 1.86	3.64 ± 0.036	0.57 ± 0.02
Hepatotoxic control	37.20 ± 1.33	1.01 ± 0.01	2.66 ± 0.05
SMHA 100 mg/kg	47.83 ± 1.39	1.25 ± 0.04	2.27 ± 0.07
SMHA 200 mg/kg	50.40 ± 1.30	1.46 ± 0.05	2.21 ± 0.07
SMHA 400 mg/kg	50.70 ± 2.33	1.86 ± 0.09	1.91 ± 0.02
SMAQ 100 mg/kg	47.05 ± 1.68	1.41 ± 0.04	2.00 ± 0.06
SMAQ 200 mg/kg	50.90 ± 2.10	1.89 ± 0.04	1.82 ± 0.06
SMAQ 400 mg/kg	54.72 ± 1.56	2.23 ± 0.10	1.68 ± 0.05
Silymarin 150 mg/kg	52.83 ± 1.92	3.13 ± 0.068	1.68 ± 0.03

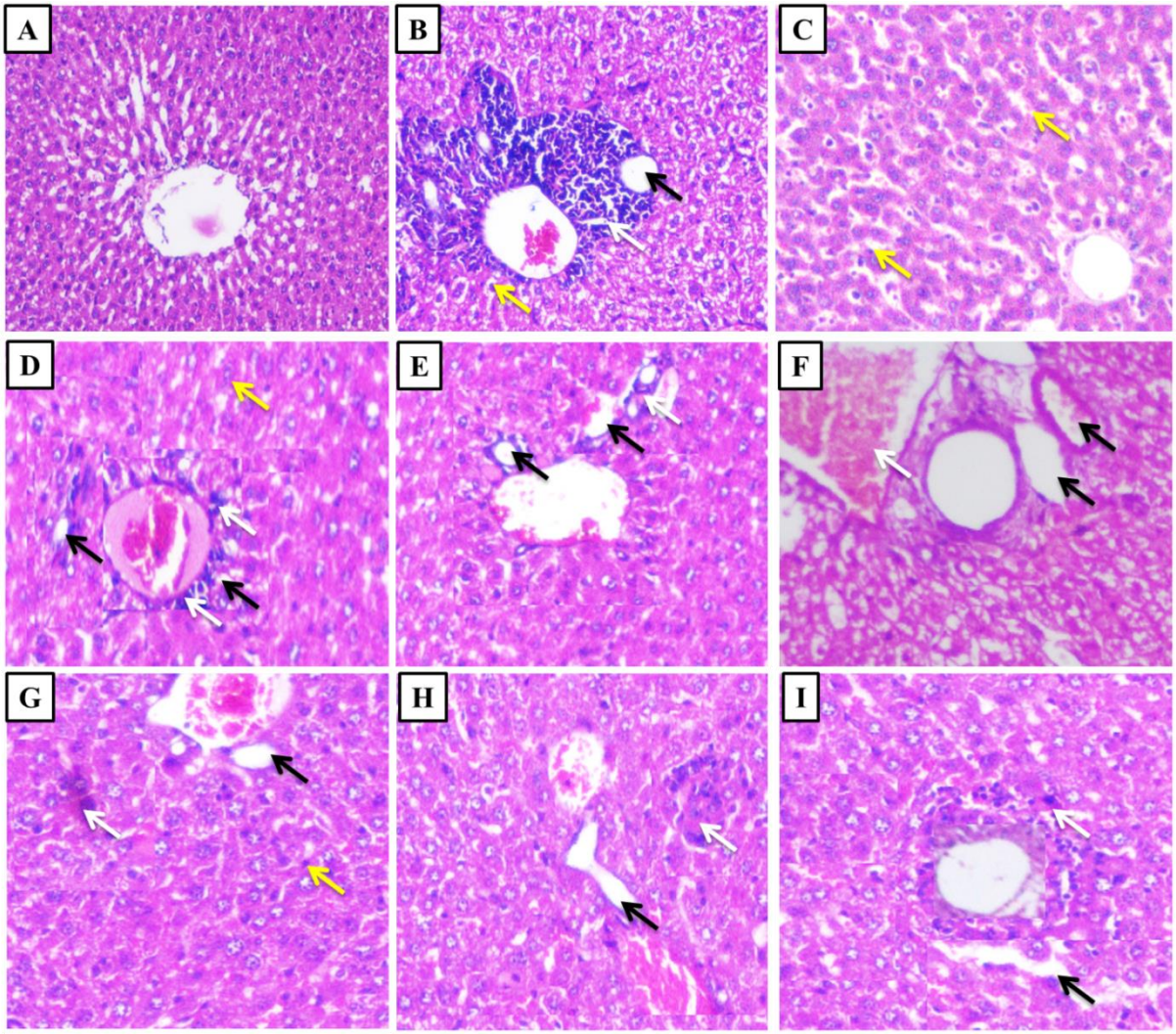

Figure 7 Histopathological changes in liver of experimental rats (H&E × 100). (A) Normal, (B) hepatotoxic control PCM, (C) Silymarin 150 mg/kg, (D) SMHA 100 mg/kg, (E) SMHA 200 mg/kg, (F) SMHA 400 mg/kg, (G), SMAQ 100 mg/kg, (H) SMAQ 200 mg/kg and (I) SMAQ 400 mg/kg [White arrows indicate inflammation; Yellow arrows indicate condensed nuclei of cells; Black arrows indicate fat infiltration]

Histopathology studies

In the livers of normal control rats, normal central vein with radiating sinusoid cords were observed. Sinusoid congestion, swelling and necrotic cells were not observed. Hepatotoxic control rats demonstrated perivenular inflammatory collection and hyperplasia of Kupffer cell with condensed nuclei and fatty infiltration. These pathological changes were alleviated by different doses of *S. melongena* extracts (figure 7).

4. DISCUSSION

Liver being the largest organ of body and also most important site of metabolism, it's highly affected by toxic agents generated post metabolism or from pro-drugs. Hence liver enzymes serve as biomarkers to detect its normal functionality. These are very sensitive to even minor toxicity which serves as a great tool in the assessment of hepatocellular damage. Serum biochemical estimation (ALT, AST, ALP) and bilirubin are the most important biomarker to test liver injury. In this study, all the biomarker enzymes were elevated signaling cellular leakage and downgrade of functional integrity of liver cell membrane (Sabiu et al., 2014). Animals when dosed with SMHA and SMAQ against PCM induced hepatoxic rats, there was observed marked attenuation in the elevated liver biomarker enzymes (AST, ALT, and ALP) and the bilirubin level. Recovery toward normalization of the enzymes following SMHA and SMAQ treatment, demonstrated that anti-oxidants in the peel especially Nasunin exhibits excellent hepatoprotective properties that was proven by histpathology. Restoration of normal membrane structure, sinusoids size, reduction in fatty cells infiltration, and healing of hepatic parenchyma proved that brinjal peel extracts have strong anti-oxidant activity and exert hepatoprotective action *in-vivo* too (Ahmed *et al*., 2001).

In SMHA and SMAQ treated animals, there was an increase in SOD activity, which could be attributed to glutathione peroxidase (GPx) activity that reduced H2O2 level, thereby preventing the retroinhibition on SOD (Kamraj *et al*., 2007). Nasunin, an anthocyanin is predicted to possess a strong free radical scavenging activity and protection against lipid and protein oxidation, which have been primarily attributed to its flavonoid fraction (González-Gallego *et al*., 2014).

5. CONCLUSION

From the study, it can be concluded that the peel extracts of *S. melongena* purple coloured fruit has promising liver protective effect as established with improvement of biochemical parameters supported with histopathological evidences. Nasunin is reported and also established in this study to be the most important and with highest anti-oxidant potential in the egg plant fruit peels. This finding lets us to postulate that Nasunin may pave path for a new strategy to prophylactically treat PCM induced liver injury. Therefore, the fruits of *S. melongena* can be used as a potential food supplement in form of functional food for both protective and reparative sources for hepatocytes.

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Author's contribution

Concept and idea by: Professor (Dr.) Nitin Kumar,
Method design by: Assistant Professor (Dr.) Pankaj Gupta,
Method review by: Professor (Dr.) Nitin Kumar and Assistant Professor (Dr.) Pankaj Gupta.

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