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LEMON PEEL (*CITRUS LIMON L.*) AS POTENTIAL SOURCE OF HESPERIDIN: AN ANTIOXIDANT POTENTIAL OF HESPERIDIN VIA SUPPRESSING MDA (MALONDIALDEHYDE) IN SODIUM ARSENITE-INDUCED HEPATOTOXICITY

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Abstract

Present study was conducted to check the effect of lemon peel extract (LPE) against SA (Sodium Arsenite)-induced liver toxicity that results in dysfunction of liver due to increase in oxidative stress. HPLC analysis with C18 column was used to analyze hesperidin content in lemon peel at 280nm. Antioxidant potential of hesperidin was analyzed via efficacy for this purpose. Sprague dawely rats were divided into 5 groups (n=30). Liver toxicity was induced by consuming 10 mg sodium arsenic per kilogram of rat body weight. Different concentrations of LPE were introduced to each experimental unit. Effectiveness of doses were analyzed by biochemical parameters. Blood samples were collected at different intervals to estimate the serum ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), MDA (Malondialdehyde), SOD (Superoxide dismutase), CAT (Catalase) and GPx (Glutathione peroxidase) level. Lemon peel extract had 16.5430 mg/100g hesperidin. Serum ALT (38.16±1.25 U/L), AST (31.67±3.06) and MDA (2.68±0.01) level was significantly lowered after LP extract consumption as compared with SA-induced liver toxicity group (38.16±1.25), (97.16±4.58) respectively. While serum SOD (163.17±1.25 U/mL), CAT (112.0±1 U/mL) and GPx (3.12±0.017 MU/L) level was significantly higher after LP extract consumption as compared with disease group (78.00±0.50 U/mL), (74.0±1.0 U/mL) and (0.83±0.04 MU/L) respectively. Present study demonstrated that lemon peel has potential to attenuate SA-induced liver toxicity and oxidative stress that directly correlated to its pharmacological properties based on polyphenol (especially hesperidin dominant flavonoid) content. Lemon peel extract has great potential against oxidative stress and related disorders. There is dire need to attain an appropriate method for extraction of lemon peel extract to get its maximum benefits.

Keywords: Aspartate aminotransferase, Catalase, Glutathione peroxidase, Malondialdehyde, Superoxide dismutase.

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1. INTRODUCTION

Lemon (*citrus limon*) is the most important cultivated plant in Pakistan and belongs to the Rutaceae family. *Citrus limon* tree (2.5-3m of length) has evergreen leaves and edible fruit in yellow color, grown preferably in a loamy, moist and well drained place. Report by Rafiq *et al.,* 2018 out of 82 million tons citrus production, about 50 million tons were used for the commercial purpose in which 44% peel is subjected as wastage. In Pakistan, lemon production is 4.19% annually. In 2018 lemon production in Pakistan was 2.25 million tons.

The USA is the largest producer of lemon after Italy, Brazil and Spain. Nowadays, China is gradually rising in lemon production and export worldwide with estimated production of lemon around 41.9 million tons, accounting for 27.52% of total production of lemon in all the world. Top 5 countries that produce lemon in large quantities are Brazil, India, Spain, Mexico and the USA. Ancient civilization used lemon juice as a medicine, but recent multiple scientific studies support lemon for various purposes to cure illness and improve health quality, considered as potential source of polyphenols and flavonoids (Klimek-Szczykutowicz *et al.,* 2020).

Flavanone is a unique class of flavonoid composed of three aglycones (naringenin, hesperidin and eriodictyol) (Kuntic *et al.,* 2014). The current research is based on flavonoids with special attention given to the most promising bioactive component (hesperidin). Hesperidin chemically known as 3, 5, 7-trihydroxy-4-metoxy-flavanone, is a glycoside flavanone (flavonoid form that is lacking in sugar moieties). Glycosides are naturally occurring compounds that have carbohydrates backbone linked with a non-sugar part through a unique linkage (may be through nitrogen, sulphur and carbon) named as glycosidic bond. The Aglycone part of glycosides are biologically active. Glycosides possess pharmacological activities due to its aglycone part.

Hesperetin (metabolite form of hesperidin) has a molecular weight of 610.57 Da. and it's found in the food in the form of Hesperidin (3, 5, 7- trihydroxyflavone 7-rhamnoglucoside). Hesperidin aglycone attaches to rhamnose sugar and glucose at 7^{th} position of a ring. It is also known as Vitamin P. Hesperetin was first discovered by French chemist Leberton in 1828 from albedo of orange and lemon.

Hesperetin is estimated to be the most highly consumed flavonoid that is equivalent to 30% (28.3mg/day) of the total daily flavonoid intake. Citrus fruits are used worldwide in a raw state. Citrus peels act as a waste discard in the environment. Citrus peel contains essential secondary components like polyphenols that have substantial anti-oxidant activities (Knekt *at al.,* 2002).

Lemon fruit and peel have pharmacological properties due to having polyphenols like flavonoids (hesperidin and naringin), flavones (diosmin and apigenin) and flavonols (quercetin). Hesperidin is the most dominant glycosylated flavonoid in citrus peel. Citrus peel is a good source of polyphenols, antioxidants and essential oil like limonene. Hesperidin is already reported as a potential element for alleviating oxidative stress and

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exerting pharmacological properties against inflammation, hepatotoxicity, diabetes and oxidation (Zoghloul *et al.,* 2017).

The present work was designed to analyze the anti-oxidant and anti-hepatotoxicity effect of hesperidin in rats with sodium arsenite-induced hepatotoxicity. Arsenite is considered a toxic metal that has the potential to cause toxicity in humans. Sodium arsenite is one of the dangerous arsenic inorganic compounds that have potential to cause cardiac, renal, hepatic dysfunction. Arsenic has shown an increased level of ROS production at molecular and cellular level especially hydrogen peroxide and superoxide. It's previously reported that plant-based flavonoids and biologically active compounds have potential to attenuate oxidative stress induced by arsenic and have gained much importance with low side effects.

In present work, lemon peel extract was used as a potential source of hesperidin and was introduced into rats to check the effect of hesperidin against sodium arsenic-induced liver toxicity and oxidative stress. In this scenario, blood serum and liver samples were analyzed biochemically to assess the reduction of oxidative stress in response to hesperidin consumption.

2. MATERIAL AND METHODS

Ethanol, hesperidin ≥80%, HPLC gradient water, 0.1 O-phosphoric acid, methanol, sodium arsenite, reagents and all chemicals used for extraction and biochemical analysis were purchased from fisher chemical and Sigma Aldrich (USA) Company.

2.1 Preparation of sample: Fresh lemons (*Citrus limon "Eureka*") were obtained from the fruit orchard University of Agriculture Faisalabad. Small pieces of lemon peel were placed into the dehydrator for 10 hours under controlled temperature and humidity. All the lemon peels were taken from the dehydrator and were subjected to grinding using a blender**.** Dried lemon peel was turned into fine powder and stored in a plastic bag at room temperature (Agarwal *et al.,* 2012).

2.2 Extraction of lemon peel (LP): Lemon peel (LP) was extracted with 75% aqueous solution of ethanol. For extraction through soxhlet apparatus, 800 mL of solvent was used. 250 g dried lemon peel was pinned up into filter paper and placed into an extraction sleeve.

The solvent was placed into a round bottom flask and attached to the apparatus. Heat was applied to move the solvent through the condenser and the pinup dried lemon peel sample to obtain extract.

This method was repeated 5 times to obtain maximum extraction. Extract was transferred from round bottom flask into air tight jars and saved for further drying. The extract was concentrated by using a rotary evaporator in accordance with Studo *et al*. (2009). Extract was concentrated under reduced pressure at 45°C by keeping it in a rotary evaporator for 4 hours.

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2.3 Characterization of hesperidin: Quantification of hesperidin was carried out by using high performance liquid chromatography (HPLC) by following the procedure described by Li *et al,* (2019) with slight modification. Analysis was done on Shimadzu HPLC (model no. CTO_20AC, with CAT no. 228_45010_32) that was equipped with lab solution HPLC software. A C18 column (Roc C18 5µm 150 × 4.6mm) with serial number 19042147 was used.

Column temperature was maintained under 30°C and hesperidin was monitored at 220 nm via UV visible detector. Mobile phase was prepared by using phosphoric acid (0.1%) and HPLC gradient water in 50:50 ratio. About 15 µl sample volume was injected and ran for 10 min. The flow rate of mobile phase was kept 1ml/min and each sample run had 3 technical replicates.

2.3.1 Sample preparation: Lemon peel extract was filtered by using 0.2 µm SFCA (Cat no. 723-9902). Sample was diluted in solvent containing water and 0.1 O-phosphoric (50:50) and filtered into a vial just before running on HPLC.

2.3.2 Stock solution and standard solution preparation: Stock solution of 1mg/ml hesperidin standard (hesperidin ≥80% pure) was prepared in water: methanol (50:50) solvent. Stock solution was used to prepare standard solutions in three different concentrations: 20, 60, and 100 µl/mL.

2.4 Efficacy trial and Disease induction: Efficacy trial was designed to check the effect of different concentrations of lemon peel extract on hepatotoxicity. For this purpose, 6-weeks old Sprague Dawley rats, weighing between 150-160 g, were used for efficacy trials due to having an excellent defensive system. The rats were procured from National Institute of Health, Islamabad and placed in the animal house of NIFSAT. Humidity levels at 45-55% were maintained.

The whole efficacy trial was approved by IBC (Institutional Biosafety and Bioethical Committee of UAF with the Reference no 1004/ORIC/23-02-2021. Hepatotoxicity was induced by sodium arsenite (Sigma Aldrich with S7400 catalog no, purity >90%) 10 mg/kg of body weight. Sodium arsenite dose to induce hepatotoxicity was based on Dash *et al*. (2018).

2.4.1 Experimental Design: Rats were divided into 5 groups and each group carried out 6 rats.

Group 1: Normal group given normal diet for 60 days

Group 2: Diseased group fed with normal diet for 60 days

Group 3: Diseased group fed with normal diet + 250 mg/kg lemon peel (LP) extract for 60 days

Group 4: Diseased group fed with normal diet + 500 mg/kg lemon peel (LP) extract for 60 days

Group 5: Diseased group fed with normal diet + 750 mg/kg lemon peel (LP) extract for 60 days

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2.5 Blood sampling: During the efficacy trial blood samples and liver samples from each experimental unit were collected at different intervals 7, 15, 30, 45 and 60th day to analyze the biochemical parameters.

2.6 Liver function analysis: SGPT/ALT and SGOT/AST levels were analyzed from each group according to the procedure described by Zareei *et al*. (2017). SGPT/ALT and SGOT/AST both enzymes are present in the liver cytoplasmic region and involve metabolic processes via actively participating in energy conversion pathways from food. These biomarkers indicate disturbance in the liver through altering their normal range. ALT and AST analysis was done by observing the oxidation reaction of NADH into NAD^+.

2.7 Assessment of ALT and AST: Analysis of liver enzymes (ALT and AST) was performed with blood serum. Firstly, the serum was separated via centrifugation at 3000 rpm for 10 min. Serum was collected into test tubes for further analysis. Reaction mixture was prepared for ALT by adding all reagents including 61 mM L-alanine (2.3 ml), 4.2 mM NADH (0.1 ml), 3.4 mM pyridoxal phosphate, 72000 U/L lactate dehydrogenase and 225 mM oxoglutarate. About 0.2 ml serum was added to the reaction mixture and incubated for 2 min at room temperature. Absorbance was analyzed by using UV Visible spectrophotometer (Evolution 260 BIO Thermo Scientific). Same procedure was repeated for AST but aspartate and malate dehydrogenase (36000 U/L) was added instead of alanine and dehydrogenase.

2.8 Assessment of oxidative stress: The body has a vast and complex defensive system that depends on endogenous antioxidants. These antioxidants may be enzymatic or non-enzymatic and differentiated by categorizing into 1st, 2nd, 3rd and 4th line defensive systems. The first line defensive system included SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase). These antioxidants have potency to neutralize the molecules that may lead to production of free radicals and participate in producing oxidative stress.

2.9 Superoxide dismutase (SOD) analysis: SOD analysis was done by following the procedure described by Tirkey *et al*. (2005) with minor alteration according to him firstly liver sample was collected and perfused on to cold saline that contains 0.09% sodium chloride after that homogenized with 1.17% potassium chloride and centrifuged at 800g at 4 °C for 5 minutes in order to separate nucleus debris part, right after to get post mitochondrial supernatant (PMS) remaining mixture was again centrifuged for 20 min at 10,500 g. stored the supernatant for further SOD analysis.

In the second step SOD values were measured by observing the auto-oxidation of hydroxylamine. Nitro blue tetrazolium (NTB 96mM), ethylenediaminetetraacetic acid (EDTA 0.01 Mm), sodium carbonate 50 mM was mixed and formed a mixture. 2ml above mixture was taken into the cuvette to add 0.5 ml Post mitochondrial supernatant and 0.05 ml hydroxylamine was added into the cuvette. SOD value was observed by checking the absorbance of mixture at 570 nm via Spectrophotometer (Evolution 260 BIO Thermo Scientific).

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2.10 CAT catalase analysis: Catalase activity was measured by following colorimetric method described by Sinah (1972) via using Cat. No KT-711. Liver was homogenized into a phosphate buffer (0.1 M) and subjected to centrifugation at 300 rpm. Supernatant was stored till further analysis. Liver sample was diluted with an assay buffer then added into the well. H_2O_2 was added into the plate and well and subjected to incubation for 30 min at room temperature. After that detection assay was added to dilation horseradish peroxidase enzyme and further incubated for 15 min. in the presence of hydrogen peroxidase enzyme substrate react with horseradish per-oxidase and gives pink color. The pink color was read at 560 nm. Decreasing level of catalase directly proportional to increasing level of hydrogen peroxidase in solution and gives pink color. Value of catalase was expressed in U/mL.

2.11 GPx (Glutathione peroxidase) analysis: Glutathione activity was by following the procedure described by Richard *et al*. (1976). Liver was perfused under sodium chloride (1.14 M) and subjected to homogenization in sucrose (0.25 M) and centrifuged at 105,000 g for 60 min.

Reaction mixture contained EDTA (1mM), NADPH (0.2 mM), GSSG-reductase (1.E.U/ml), GSH (1 mM), NaN_3 (1 mM) and H_2O_2 in 1 mL volume. Mix it well and enzyme sauce was added into mixtures and subject to incubation for 5 min at room temperature. Absorbance was analyzed at 340 nm via Spectrophotometer (AA 240, Varian, Australia).

2.12 Serum MDA level: The Serum Malondialdehyde (MDA) level of each subject was analyzed by following the procedure that was previously described by D'souza *et al.* (2012). Blood sample of each subject was collected and subjected to serum separation process by centrifugation of blood sample at 3000 rpm at least for 10 min, after separating the serum (100 µL) diluted by using distilled water and made upto 500 µL volume. Diluted sample was kept in boiling water for 15 minutes after that chemicals including trichloroacetic acid TCA-2- thiobarbituric acid- HCL reagent upto 1 ml was added into diluted sample and subjected to cooling. After that sample was centrifuged at 3000 rpm to separate the supernatant. Reaction between sample and thiobarbituric acid gives pink color and reads its intensity at 535 nm by using spectrophotometer (Evolution 260 BIO Thermo Scientific), intensity of pink color shows MDA level in sample. Greater the intensity directly proportional to MDA level.

2.13 Statistical analysis: To achieve the maximal information data will be collected from all experimental units that are randomly allocated and subjected to regression analysis to assess the level of significance ($\alpha \leq 0.05$). To check the effect of multiple variables on different parameters CRD (Factorial under complete randomized design) will be used (Montgomery, 2008).

3. RESULTS

3.1 Lemon peel extraction: Lemon peel extraction was done through ethanol to analyze the volatility efficiency of solvent, for this purpose solvent was used in the concentration about 75%. Ethanol is known as a good volatile compound with its molecular weight about

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46.07 g/mol and used for botanical extraction. Ethanol is considered a good solvent for phenolic content analysis. Soxhlet apparatus was used for extraction preparation by following the procedure previously described WFD by Sharma *et al.,* (2013). Extract was concentrated by using a rotary evaporator and used for hesperidin characterization.

3.2 Characterization of Hesperidin in lemon peel extract: Hesperidin content was measured by using C18 column (Ros C18 5µm 150× 4.6mm) with mobile phase of water: O-phosphoric acid at 220 nm wavelength. LC (liquid chromatography) and PDA (photodiode-Array) detection was measured to analyze the concentration of hesperidin in standard and lemon peel samples has been shown in (Fig 1 and 2).

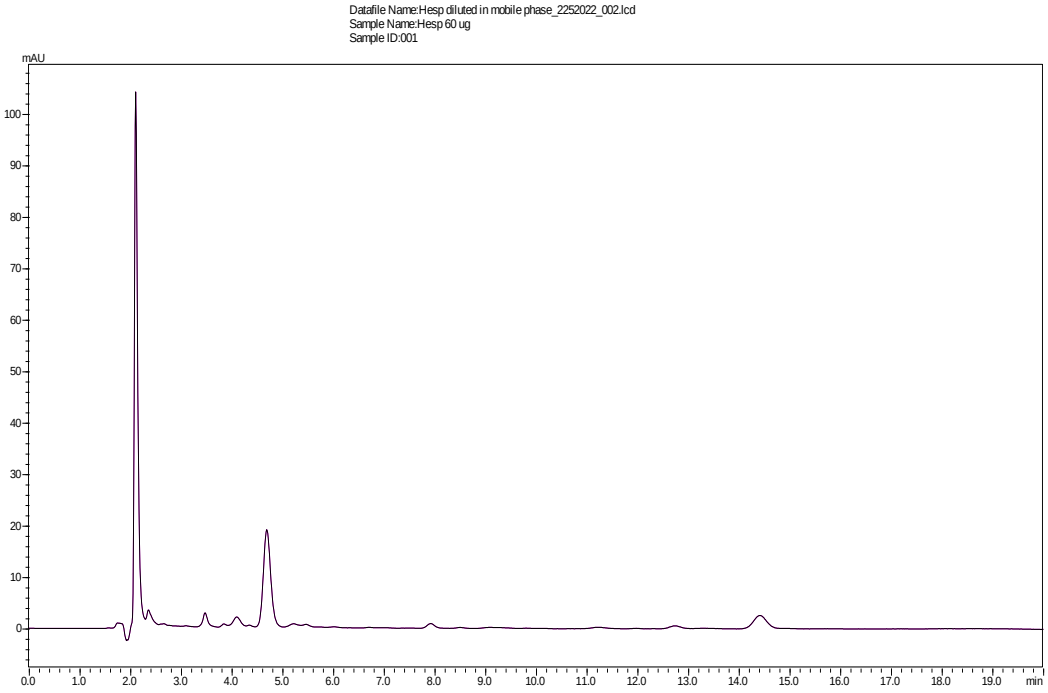

Figure 1: HPLC chromatogram of hesperidin standard

3.2.1 Validation method: Retention time and peak area of standards was used to analyze the exact concentration of hesperidin in lemon peel extract samples. Calibration curve was generated by using peak area and volume of standards as shown in (Fig. 3)**.** And calibration curve showed good linear regression (Y= 3337.9X + 38989) this calibration curve was used to quantify hesperidin in lemon peel extract. Peak was generated at 4.6 min (retention time). Calibration data has been mentioned in (Table no 1).

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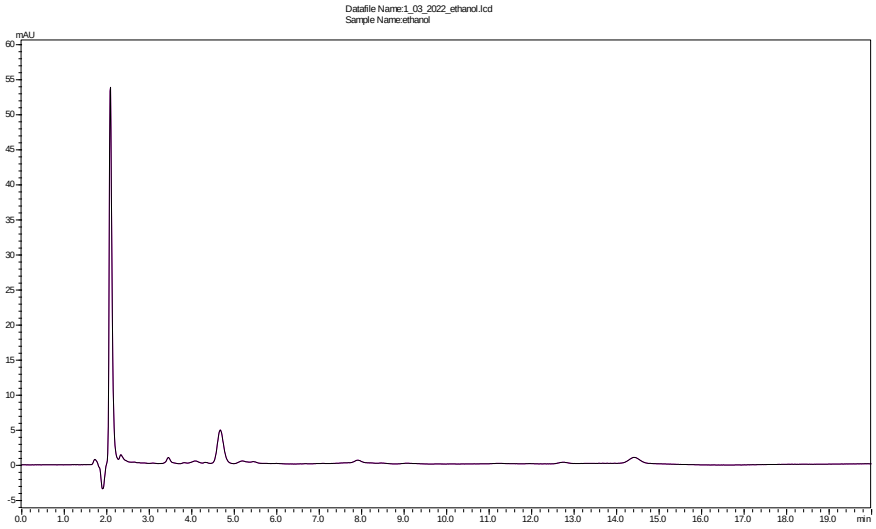

Figure 2: HPLC chromatogram of ethanol extract of lemon peel (*citrus limon* L.)

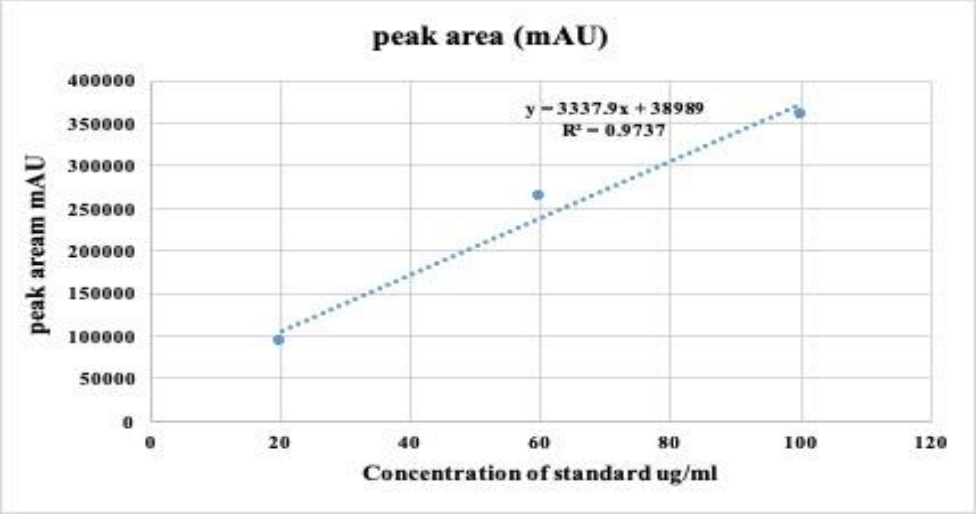

Figure 3: Hesperidin standards calibration curve

Table no 1: HPLC Proposed calibration curve

Active compound	Concentration (µg/ml)	Area of peak (mAU)
Hesperidin	20	93086
	60	264589
	100	360121

Hesperidin content in lemon peel extract was quantified by comparing the retention time with standards and found that lemon peel ethanol extract showed concentration was about 16.5430 mg/100g. It was found that ethanol has higher curing potential of hesperidin from lemon peel.

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3.3 Impact of Lemon Peel (LP) extract on ALT: ALT level was analyzed to check the impact of lemon peel (LP) extract. ALT level was significantly increased in Sodium arsenite-induced hepatotoxicity rats as compared with the control group. LP extract treatment for 2 months showed a significant effect on ALT level**.** Mean of ALT level of each group are shown in (Table 2) shows that after LP extract consumption serum ALT level was significantly reduced. Trend of decreasing ALT level found in order to T3>T2 >T1 treated with 750, 500 and 250 mg/kg LP extract. 750 mg/kg LP extract consumption showed significant reduction as compared with other concentrations due to high content of hesperidin Flavanone that exerts anti-hepatotoxicity effects by modulating altered levels of ALT (Fig 4).

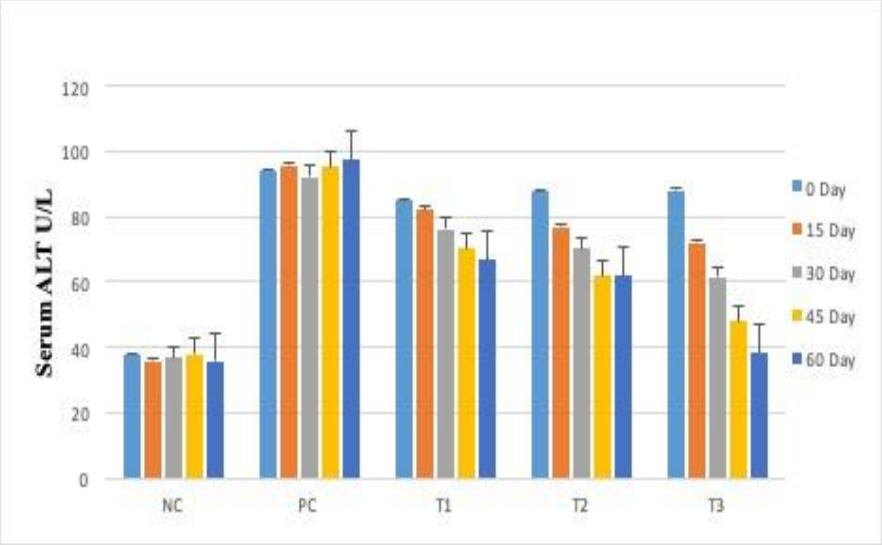

Figure 4: Effect of different doses of lemon peel extract on serum ALT U/L level of liver

All values are expressed in mean ± S.D. NC*= normal rats, PC*= SA-induced liver toxicity rats, T1* = SA-induced + 250 mg LPE, T2*= 500 mg LPE, T3* = 750 mg LPE. Serum ALT level was significantly higher in the diseased group as compared with normal group. Higher dose 750 mg/kg lemon peel extract showed significant ($p < 0.05$) effects on serum ALT level.

3.4 Impact of Lemon Peel (LP) extract on AST: Sodium-arsenite caused marked changes in AST level of treatment groups as compared to normal groups. LP extract consumption showed significant reduction in AST level as compared with normal groups. Mean of AST Level of each group are mentioned in (Table 2) that shows significant reduction in AST level after LP extract consumption. Reduction trend was found in order to T3>T2 >T1 treated with 750,500 and 250 mg/kg LP extract. 750 mg/kg LP extract consumption has the most significant effect on AST level as compared to 500 and 250 mg/kg LP extract because hesperidin performs biological activity as anti-oxidant and ameliorates hepatic toxicity by reducing serum AST level (Fig 5).

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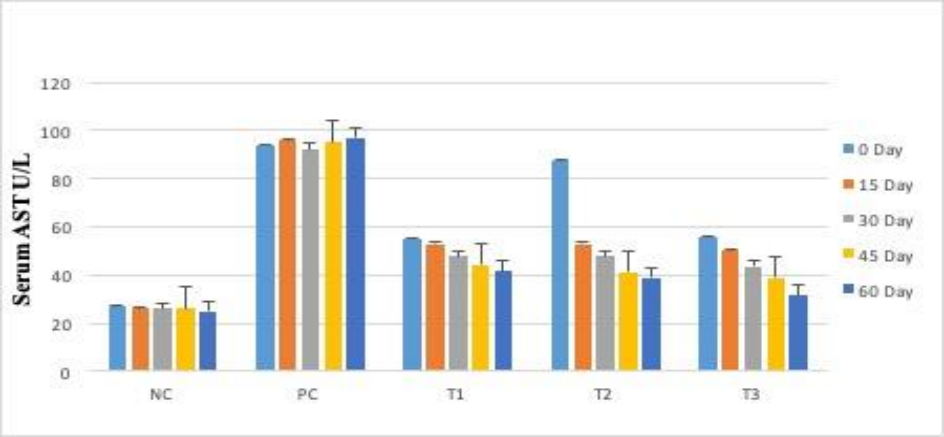

Figure 5: Effect of different doses of lemon peel extract on serum AST U/L level of liver

All values are expressed in mean ± S.D. NC*= normal rats, PC*= SA-induced liver toxicity rats, T1* = SA-induced + 250 mg LPE, T2*= 500 mg LPE, T3* = 750 mg LPE. Serum AST level was significantly higher in the diseased group as compared with normal group. High dose 750 mg/kg lemon peel extract exerted significant ($p < 0.05$) effects on serum AST level.

3.5 Impact of Lemon Peel (LP) extract on Serum Superoxide dismutase (SOD): SOD level was significantly decreased after disease induction compared to the normal group. Means values for SOD level of each group after LP extract consumption are depicted in (Table 2) shows significant increase in SOD level. Increasing trend of SOD level was found in order to T3>T2 >T1 group. 750 mg/kg LP extract consumption was found more effective and significantly changed the altered level of SOD. Hesperidin content in lemon peel extract has antioxidant potential and maintains a first line defensive system via enhancing SOD level (Fig 7).

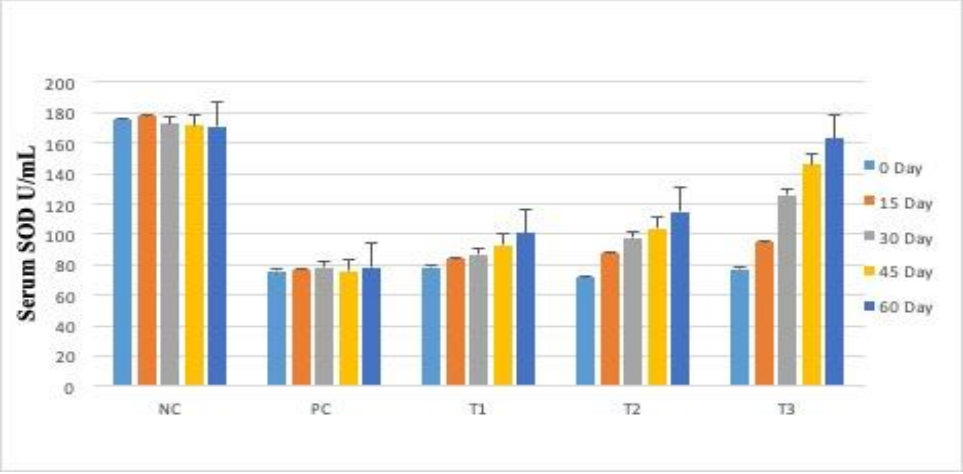

Figure 7: Effect of different doses of lemon peel extract on serum SOD U/mL level of liver

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All values are expressed in mean ± S.D. NC*= normal rats, PC*= SA-induced liver toxicity rats, T1* = SA-induced + 250 mg LPE, T2*= 500 mg LPE, T3* = 750 mg LPE. Serum SOD level was significantly lowered in diseased group as compared with normal group.750 mg/kg lemon peel extract exhibited significant ($p < 0.05$) effects on SOD level after oral administration of 60 days.

3.6 Impact of Lemon Peel (LP) extract on Serum Catalase: Catalase level was decreased after disease induction compared with the normal group. Mean values for CAT of each group are mentioned in (Table 2) showed that LP extract consumption for 60 days brings dramatic change in catalase enzyme level and level was reversed to its normal value after consumption of LP extract as compared with diseased group. Increasing trend of catalase level was found in order to T3>T2 >T1. 750 mg/kg LP extract consumption showed more effectiveness against oxidative stress by exerting free radical scavenging activity of hesperidin and ameliorates oxidative stress via enhancing CAT level **(**Fig 8).

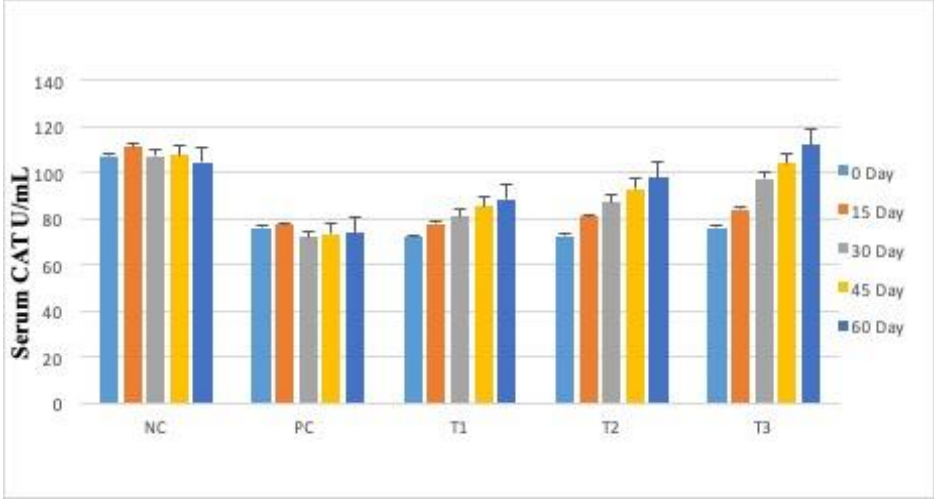

Figure 8: Effect of different doses of lemon peel extract on serum CAT U/mL level of liver

All values are expressed in mean ± S.D. NC*= normal rats, PC*= SA-induced liver toxicity rats, T1* = SA-induced + 250 mg LPE, T2*= 500 mg LPE, T3* = 750 mg LPE. Serum CAT level was significantly lowered in the diseased group as compared with normal group. Higher dose 750 mg/kg lemon peel extract showed significantly ($p < 0.05$) effects on CAT level.

3.7 Impact of Lemon Peel (LP) extract Serum Glutathione peroxidase (GPx): GPx value was significantly decreased after disease induction as compared with the normal group. Means values for GPx are described in (Table 2) shows significant increase in GPx level as compared with diseased group. Increasing trend was found in order to T3>T2 >T1. Effect was higher in high dose as compared to low dose. 750 mg/kg LP extract consumption showed significant ability to reduce oxidative stress due to hesperidin flavonoid and activates defensive mechanisms at cellular level by scavenging free radicals via up-regulation of GPx level **(**Fig 9).

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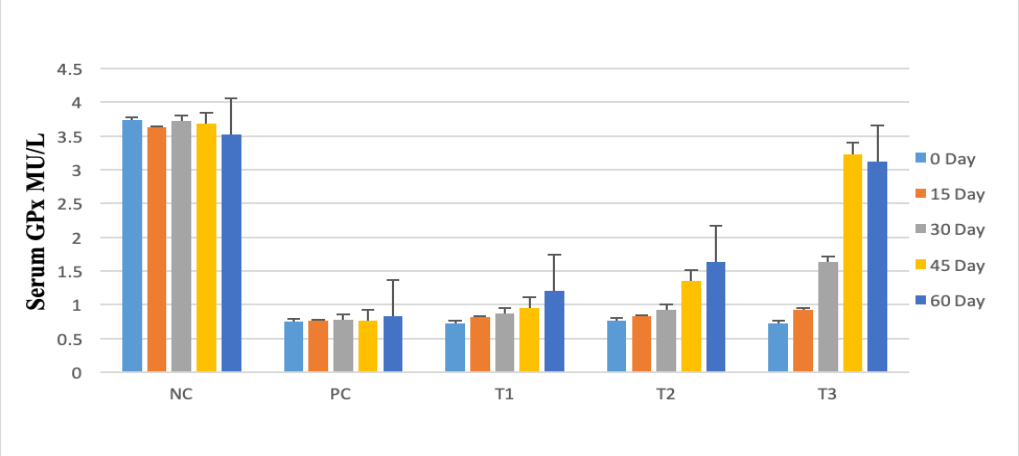

Figure 9: Effect of different doses of lemon peel extract (LP) on serum GPx MU/L level of liver

All values are expressed in mean ± S.D. NC*= normal rats, PC*= SA-induced liver toxicity rats, T1* = SA-induced + 250 mg LPE, T2*= 500 mg LPE, T3* = 750 mg LPE. Serum GPx level was significantly lowered in diseased group as compared with normal group.750 mg/kg lemon peel showed significant ($p < 0.05$) effects on GPx level as compared with disease group.

3.8 Impact of Lemon Peel (LP) on Malondialdehyde (MDA): Serum MDA level was significantly higher after disease induction that indicates low level of antioxidant in response to liver toxicity. Hesperidin consumption is found to have potential to manage oxidation in disease conditions. Mean square values have been depicted in (Table 2) that described the ability of different hesperidin doses against oxidation. MDA level was significantly reduced after hesperidin consumption up to 60 days, an effective decreasing trend was found in group T3 that was administered with 750 mg/kg hesperidin extract for 60 days (Fig 6).

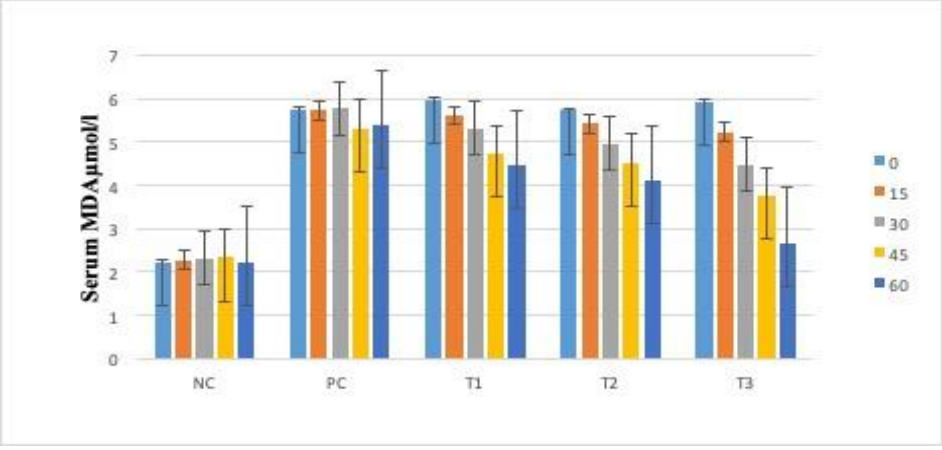

Figure 6: Effect of different doses of lemon peel extract (LP) on serum MDA µmol/l level

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Table 2: Effect of Lemon peel (LP) extract on biochemical parameters against (SA) Sodium Arsenite –induced liver toxicity

Parameter	control group	SA-induce group	SA+250 LPE	SA+500 LPE	SA+750 LPE
ALT U/L	35.83 ± 0.76^{L}	97.16 ± 0.76^{A}	66.83 ± 0.76^{I}	62.00 ± 1.00^{J}	38.16 ± 1.25^{L}
AST U/L	25.67 ± 2.52^{I}	97.16 ± 4.58^{A}	41.67 ± 2.08^{DEFG}	38.67 ± 3.51^{FGH}	31.67 ± 3.06^{GHI}
SOD U/mL	171.00 ± 1.00^{C}	78.00 ± 0.50^{NO}	100.83 ± 1.04^{I}	115.00 ± 1.00^{G}	163.17 ± 1.25^{D}
CAT U/mL	104.67 ± 1.53^{BC}	74.0 ± 1.0^{KLMN}	88.33 ± 0.58^{F}	98.00 ± 1.00^{D}	112.0 ± 1^{A}
GPx MU/L	3.52 ± 0.01^{C}	0.83 ± 0.04^{K}	1.21 ± 0.01^{H}	1.63 ± 0.02^{F}	3.12 ± 0.017^{D}
MDAμmol/L	2.24 ± 0.02^{QR}	5.39 ± 0.01^{F}	4.48 ± 0.01^{L}	4.12 ± 0.01^{M}	2.68 ± 0.01^{O}

All values are expressed as mean ± S.D of n=6 rat in each group. Alteration in superscripts in upper case in the same row were significant (p< 0.05) difference among the group. Each group was treated with a different concentration of lemon peel (LP) extract. Higher dose (750 mg/kg LP) showed significant effects (p < 0.05).

4. DISCUSSION

Sodium arsenite is a potent drug that is widely present in the environment as an organic and inorganic form and provokes liver toxicity by inducing oxidative stress in the form of increasing levels of ROS, H_2O_2, NO and OH radicals. Liver is the most target organ for sodium arsenic toxicity (Klibet *et al.,* 2016).

In present study administration of 750 mg/kg lemon peel extract for 60 days exhibit remarkable changes in liver toxicity by modulating ALT, AST, SOD, CAT, GPx and MDA level, after the progression of liver toxicity liver enzymes ALT and AST and serum MDA level was dramatically increased as compared with normal group that ultimately shows liver deterioration due to high level of oxidative stress.

In addition, the first line defensive system was also impaired by oxidative stress which showed by low levels of SOD, CAT and GPx. Malondialdehyde is the final product of polyunsaturated fatty acid deterioration after attack by oxidative stress and considered as a reliable biomarker of oxidative stress and lipid peroxidation. Our finding confirmed that sodium arsenite increased oxidative stress and hepatotoxicity as compared with normal groups.

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The efficacy trial in order to reduce hepatotoxicity was conducted and evaluated that on the basis of results flavonoid (Hesperidin) in lemon peel extract exhibits antioxidant potential to cure liver toxicity. Hesperidin is a biologically active substance in lemon peel extract that exerts pharmacological properties against oxidative stress and inflammation (Tripathy and Oelmuller, 2012).

Hesperidin flavanone radially available in the peel of lemon and its pharmacological properties against oxidative stress and inflammation was well documented in various studies (Gandhi *et al.,* 2009; Ouyang *et al.,* 2017). Our observation of anti-hepatotoxicity potential of lemon peel extract in different concentrations (250, 500 and 750 mg/kg) was conducted and we found a significant recovery after oral administration of 750 mg/kg lemon peel extract in liver toxicity. Serum ALT, AST and MDA was significantly lowered by reducing oxidative stress.

Findings of present study regarding ALT level was relate able with Kaur *et al.* (2006) and Tirkey *et al.* (2005) who elucidate the potential ability of hesperidin against liver damage and found note able rise in ALT level after liver toxicity compared with normal group. Reduction of AST level was supported by Tirkey *et al.* (2005) and Abd-Elhakim *et al*. (2020) who found marked reduction in serum AST level after introducing hesperidin against hepatotoxicity. Finding of MDA level was in accordance with Mesallam and Atef, (2020) according to them Hesperidin has found effectiveness against hepatotoxicity that was induced by Diazinon in albino rats. Hesperidin consumption reduced MDA level ranging from 126.8 ± 8.7 to 44.7 ± 3.4 (nmol/g tissue).

Present work was in contrast with Abd-Elhakim *et al.* (2020), who evaluated that hesperidin has potential against CCl_4 induced liver toxicity, administration of hesperidin for 4 weeks exerts positive outcomes against liver injury by reducing lipid oxidation. MDA level was found significantly reduced after 4 weeks administration of hesperidin.

MDA level was reduced ranging 48.39 ± 2.86 to 19.47 ± 1.49 nmol/mL as compared with the control group. Hesperidin potential to reduce oxidation was well documented by Turk *et al.* (2019), according to him hesperidin consumption 200 mg/kg for 15 possess antioxidant effects against sodium-arsenite induced hepatotoxicity.

MDA level was significantly reduced after introducing hesperidin, level of MDA was reduced from ranging 159.22 ± 1.42 to 76.98 ± 1.51 nmol/g tissue as compared with disease group. MDA finding of present work was relate-able with the observation of Hanedan *et al*. (2018), who demonstrated that hesperidin consumption ameliorate oxidative stress via modulating the level of MDA ranging from 77.16 ± 0.59 to 56.00 ± 0.32 nmol/g tissue when administered against colistin induced renal injury.

Increasing trend in SOD level was in accordance with Turk *et al.* (2018) and Cetin *et al.* (2014) who found that hesperidin consumption against hepatotoxic rats brings dramatic changes in liver enzymes specially SOD level was markedly increased after hesperidin consumption. Hesperidin consumption possesses an anti-oxidant effect and causes significant increase in CAT activity level.

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Finding of present study also relatable with Cetin *et al.* (2014) and Turk *et al.* (2018) who found hesperidin effective against liver toxicity and has potential to restore the CAT level as compared to diseased group. Present work also supported by Sabah *et al.* (2018), who found antioxidant potential of hesperidin. Orally consumption of hesperidin leads to significant increase in CAT level as compared to diseased group. Anti-hepatotoxicity potential of hesperidin reported by Sabah *et al.* (2018) and Turk *et al.* (2019) via modulating GPx level.

5. CONCLUSION

In conclusion, it was found that lemon peel has anti-hepatotoxicity and antioxidant effects. Lemon peel extract is a potential source of flavonoid hesperidin that ameliorates hepatotoxicity and oxidative stress via exerting its pharmacological free radical scavenging activity that ultimately has positive outcomes. Lemon peel extract gently imposes an antioxidant effect that reduces AST, ALT and MDA levels and increases antioxidants SOD, CAT and GPx level.

Increases in dose directly linked with positive effect of lemon peel extract due to increase of hesperidin content in extract. It was found that higher dose 750 mg/kg LP extract has positive outcomes rather than 250 and 500 mg/kg LP extract. On the behalf of results, it is clear that 750 mg/kg Lemon peel (LP) extract possess significant effects on liver toxicity and improve anti-oxidant status by maintaining the level of first line defensive system.

Lemon waste can be used as a potential source of flavonoids (hesperidin) to improve the quality of life. Present study concludes that lemon peel has amenable ability to attenuate liver toxicity and oxidative stress. This pharmacological effect of lemon peel can be correlated with its antioxidant property directly.

Conflict of interest

All authors declared that there is no conflict of interest

Author's contribution statements

MS and RS executed the efficacy study, TZ and BI supervised in data analysis and interpretations.

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