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Bhadarka Dixita H
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Patel Urvesh D
College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, Gujarat, India

Solanki Shivani L
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Modi Chirag M
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Patel Harshad B
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Singh VK
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Correspondence
Bhadarka Dixita H
College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, India

Antioxidant effect of poly-herbal extract mixture against paracetamol induced oxidative alterations in rats

Bhadarka Dixita H, Patel Urvesh D, Solanki Shivani L, Modi Chirag M, Patel Harshad B and Singh VK

Abstract

The present experiment was carried out to investigate the amelioration of paracetamol induced oxidative alterations by poly-herbal extract mixture (PHEM at 100, 200 and 300 mg/kg, PO) and silymarin (10 mg/kg, PO) in rats. SOD activity in serum was higher in rats treated with paracetamol whereas it was slightly lower in rats treated with either silymarin or PHEM at 200 mg/kg. The activities of SOD in liver and kidney of rats treated with paracetamol were non-significantly higher which were found lower in rats treated with higher dose of PHEM.

Blood catalase activity was found lowered in paracetamol treated rats and significantly higher in PHEM treated groups (200 and 300 mg/kg). Activity of catalase in liver of rats treated with paracetamol was highest which was almost double than the value found for rats treated with PHEM at higher dose. Catalase activity in kidney in rats under treatment was not significantly altered. Blood GSH level was not significantly altered in all groups under the study. The GSH level in liver of rats treated with PHEM (200 and 300 mg/kg) were higher than those values observed in other groups. In conclusion, administration of PHEM orally at higher doses for 21 days has shown ameliorating effect against mild oxidative alterations caused by paracetamol in rats.

Keywords: Oxidative alteration, paracetamol, poly-herbal extract mixture, rats

1. Introduction

The popularity of herbal based drugs is increasing due to its efficacy and having low or no side effects on body [1]. The oxidative stress is important pathological conditions which is responsible for damage at cellular level. Drugs of herbal origin provide rational means for the treatment of several diseases in human and animals [2]. The good therapeutic effect with patient compliance, less side effects and cost effectiveness are the reasons for choosing drugs from natural origin [3].

The liver and kidney is main organs for detoxification and play important role to maintain homeostasis in the body. Many chemicals and drugs used for therapeutic purpose cause damage to liver and kidney through various mechanisms. Oxidative damage caused by such agents creating wasting conditions in the body. It is needed to counteract oxidative stress for prevention of further damage.

The various experiments in the area of Hepatoprotective and nephroprotective effects of drugs of herbal origin have been carried out by many researchers. Important hepatoprotective plants are *Capparis decidua*, *Allium cepa*, *Sphaeranthus indicus*, *Gymnosporia Montana*, *Luffa echinata*, *Tamarindus indica*, etc. Plants having nephroprotective properties are *Boerhavia diffusa*, *Moringa oleifera*, *Andrographis paniculata*, *Aervalanata*, *Cratae vanurvula* etc. [4]. Some of above plants have shown good *in-vitro* antioxidant effect due to presence of various active constituents.

With objective to formulate and evaluate the antioxidant effect of the herbal extract mixture with special attention to liver and kidney in rats, six medicinal plants (*Luffa echinata* Roxb., *Allium cepa* L., *Capparis decidua*, *Gymnosporia Montana* (Roth) Benth., *Andrographis paniculata* (Burm. f.) Wall. Ex Nees, *Boerhavia diffusa* L.) Have been used to make poly-herbal extract mixture (PHEM) and employed in the study to evaluate its antioxidant potential against oxidative alterations caused by paracetamol in rats.

2. Materials and Methods

2.1 Experimental animals

Forty two rats (10-12 week of age) were used in the experiments which were procured from

Zydus Research Center, Ahmedabad, Gujarat. Proper guideline (CPCSEA, 2003) was followed for husbandry practices during the study. The experimental protocol was approved by the Institutional Animal Ethics Committee of the college.

2.2 Plant materials, drugs and chemicals

Leaves of *Andrographis paniculata*, *Boerhavia diffusa*, *Gymnosporia Montana*, stem of *Capparis deciduas*, fruit of *Luffa echinata* and peels of *Allium cepa* were collected from nearby area of Junagadh and scientifically identified by Mr. Punit Bhatt (Pharmacognosist, Department of Pharmacology and Toxicology, Veterinary College, JAU, Junagadh). The collected material has been subjected to shade drying and power was prepare using electric grinding. Further, they were used to prepare hydro-alcoholic extracts using double distilled water and methanol (50:50). A Whatman filter paper No. 1 was used to filter the extracts and they were concentrated using rotary evaporator. All extracts were completely dried and stored in refrigerator for further use in equal proportion to prepare PHEM. Paracetamol (Lot No.:SLBR2060V) and silymarin (Lot No.:BCBT9170) were purchased from Sigma Aldrich, USA. All other chemicals and solvents of analytical grade were purchased from Merck Ltd., Mumbai and S.D. fine Chemicals, Mumbai.

2.3 Experimental Design

As mentioned by Eliwa *et al.*^[5], paracetamol was administered to rats which could able to produce oxidative changes in body. Thirty mL sunflower was used to dissolve 6 g of paracetamol and administered orally at dose of 500 mg/kg to rats except rats of normal control and vehicle control groups. Different treatments were given to each group of six rats which were randomly divided based on body weight in seven groups. Groups of rats treated with different treatments were Normal Control (C1), Vehicle Control (C2), Toxicity Control (C3), Standard drug control (C4), Treatment 1 (T1), Treatment 2 (T2), Treatment 3 (T3) for 21 days. Silymarin as a standard drug was given at the dose rate of 10 mg/kg body weight orally for 21 days. Silymarin (30 mg) was dissolved in 3mL of distilled water. PHEM (1200 mg) was dissolved in 12 mL of distilled water and given by oral route at the dose rate of 100, 200 and 300 mg/kg daily for 21 days to animals of group T1, T2 and T3, respectively. Paracetamol, silymarin, PHEM and vehicle in different groups were administered daily using oral gavage needle as per treatment protocol mentioned above.

2.4 Collection of samples

Blood samples were collected on day 22 of experiment for evaluation of oxidative stress parameters. All rats were humanely sacrificed on 22nd day of experiment and tissues of liver and kidney were collected in phosphate buffer for evaluation of parameters of oxidative stress.

Preparation of blood lysate

Blood sample (50 μ L) was mixed with 450 μ L of RBC lysis buffer (Sigma Aldrich, Lot no. RNBG0536) and kept for 5 min for efficient erythrocyte lysis. The resultant blood lysate was used for evaluation of catalase and glutathione (GSH) antioxidant enzymes, whereas the direct serum sample was used for analysis of superoxide dismutase (SOD).

Preparation of tissue sample

Liver and kidney samples (100 mg) were collected from all

rats and immediately stored in ice cold 0.1 M (1 mL) phosphate buffer saline (PBS, pH:7.4) for evaluation of catalase and GSH, whereas liver and kidney samples (0.5 g) were separately collected in Tris-EDTA buffer (pH:8.2) for analysis of SOD. Tissue samples were homogenized followed by centrifugation at 10,000 rpm at 4 °C for 10 min and supernatant from each set of sample was used for evaluation of catalase, GSH and SOD antioxidant enzymes.

2.5 Estimation of protein content in liver and kidney tissue

Protein estimation in liver and kidney was carried out using the standard method^[6]. These data were used to calculate catalase activity in liver and kidney tissues.

2.6 SOD activity in serum sample

Serum (Cu-Zn) SOD activity was determined by a simple and rapid method based on the ability of the enzyme to inhibit the autoxidation of pyrogallol^[7]. The autoxidation of pyrogallol was investigated in the presence of Tris-EDTA at pH range 7.9-10.6. The rate of autoxidation increases with increasing pH. The autoxidation of pyrogallol in the presence of Tris-EDTA buffer at pH range 8.2-8.5 is 50%. For control reading: To 2.9 mL of Tris-EDTA buffer, 0.1 mL(20mM) of pyrogallol solution was added, mixed and reading was taken at 420 nm, exactly after 1 minute 30 seconds and 3 minutes 30 seconds. The absorbance (A) per two min difference was recorded, which shows rate of autoxidation of pyrogallol. For sample reading: To 2.8 mL of Tris-EDTA buffer, 0.1 mL of serum sample was added, mixed and started the reaction by adding 0.1 mL of pyrogallol solution (as per control). It was read at 420 nm exactly after 1 min 30 seconds and 3 min 30 seconds and absorbance (B) per 2 min difference was recorded. Units of SOD/3 mL of assay mixture was calculated from $[(A-B) * 100]/(A \times 50)$. Unit*10 = Units /mL of sample solution. One unit of superoxide dismutase is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation per 3 mL assay mixture.

2.7 SOD activity in liver and kidney tissues

Superoxide dismutase (SOD) activity in tissues was determined according to the method described previously^[7]. All tissue homogenates were prepared in Tris-EDTA buffer centrifuged for 40 min at 10000 rpm at 4°C; the supernatant was used for the enzyme assay. Tris-EDTA (2900 μ L) and 100 μ L pyrogallol (2 mM) were taken in the cuvette and scanned for 3 min at 420 nm wavelength. Tris-EDTA buffer (2890 μ L, pH-8.2), 100 μ L pyrogallol and 10 μ L of tissue homogenate were taken and scanned for 3 min at the same wavelength. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50% and was expressed as Units/mg protein/min. The enzyme unit can be calculated by using the following equations: % of inhibition = $[(A-B) * 100]/B$. Enzyme unit (U) = $(\% \text{ of inhibition}/50) * \text{common dilution factor (100)}$. 50% inhibition is similar to 1 U.

2.8 Catalase activity in liver and kidney tissues

Whole blood (50 μ L) was mixed with 450 μ L of 1x RBC lysis buffer to form blood lysate. Then 20 μ L blood lysate was mixed with 1980 μ L PBS (0.1 M PBS, pH 7.5) in a test tube. Then 1 mL of 30 mM H₂O₂ was added to it and absorbance of reaction was taken at 240 nm in a spectrophotometer for 1 min, against blank having mixture of PBS and blood lysate only without H₂O₂. Unit activity of catalase was expressed in molar/min^[8].

2.9 Catalase activity from tissue

Tissue samples (100 mg) of liver and kidney were homogenized using 1mL PBS (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 5 min. Then 20 μ L of supernatant was taken out and mixed with 1980 μ L PBS (0.1 M, pH 7.4). One mL of H₂O₂ (30 mM) was added to it and absorbance of test sample was taken at 240 nm against blank having mixture of PBS and tissue homogenate only. Activity of catalase was calculated using the molar extinction coefficient of 43.6 cm⁻¹ [8]. Mmoles of H₂O₂ decomposed/min/mg protein was calculated with formula as ($\Delta A/\text{min} \times 1000 \times 3$) / (43.6 * mg protein in sample)

2.10 GSH level in blood

Blood lysate (10 μ L) was mixed with 2970 μ L of PBS (0.1 M PBS, pH 7.5) in a test tube and dTNB (20 μ L, 30 mM) was added in to it. The mixture was allowed for reaction up to 45 min and absorbance was taken at 412 nm against blank having mixture of PBS and blood lysate only without dTNB using spectrophotometer. Concentration of GSH was expressed in molar [9].

2.11 GSH levels in liver and kidney tissues

Tissue samples (0.5 g) of liver and kidney were homogenized using 1 mL PBS (0.1 M, pH 7.4). Tissue homogenate (0.5 mL) was added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA for precipitation of the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 10,000 rpm. The supernatant (200 μ L) then transferred to a new set of test tubes and added with 1.8 mL of the Ellman's reagent (5, 50-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.1 M phosphate buffer with 1% of sodium citrate solution). All test tubes were made up to the volume of 2 mL. After completion of the total reaction, absorbance was measured at 412 nm against blank having mixture of PBS and supernatant. Absorbance values were compared with a standard curve generated from known concentration of GSH [9].

2.12 In-vitro detection of flavanoids and phenolic contents in extracts

HPTLC applicator Linomat 5.0 (Camag, Germany) with software WinCAT 2.0 was used for thin layer chromatography

analysis of extracts. Each extract (100 mg) was dissolved in 5 mL of methanol with sonication for 5 minutes. Mixture was centrifuged at 5000 rpm for 5 min. Upper layer was used to detect the flavonoids and phenolic compounds in the extracts. Quercetin, rutin and gallic acid were dissolved in methanol (5 mg/10 ml) and used as reference standard. Five μ L of standards and extract solution were applied on TLC plates (Silica gel GF₂₅₄, Merck, Germany). After application of the test compounds, the plates were allowed to elute the compounds with different mobile phases (after saturation time of 1 h) up to 8 cm path as mentioned below. Mobile phase for quercetin, rutin and gallic acid were n-hexane: ethyl acetate: glacial acetic acid (31:14:5), ethyl acetate: formic acid: Glacial acetic acid: water (100:11:11:26) and toluene: ethyl acetate- formic acid: methanol (6:6:1.2:0.25), respectively [10, 11]. Derivatization was done with natural product polyethylene glycol reagent (1% methanolic diphenyl boric acid β -ethylamino ester, followed by 5% polyethylene glycol 4000). The plates were observed for bands of flavonoids and phenolic compounds in the samples. Rf value for each separated compound was calculated as per standard method.

2.13 Statistical analysis

All the data obtained were presented as means \pm standard error (SE). Data were analyzed statistically by one way ANOVA and different treatment group means were compared by Duncan's Multiple Range Tests to observe difference among the treatments [12].

3. Results

Effects of daily oral administration of poly-herbal mixture (100, 200 and 300 mg/kg, p.o.) and silymarin (10 mg/kg, p.o.) for 21 days on SOD, catalase and GSH were evaluated in the study. Mean values of serum SOD activity were 8.67 \pm 0.85, 6.67 \pm 0.55, 11.05 \pm 0.79, 9.52 \pm 1.06, 11.62 \pm 0.64, 10.10 \pm 1.01 and 11.81 \pm 0.58 U/mL in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively. Mean values of liver and kidney SOD activity were 44.00 \pm 10.48, 44.00 \pm 4.62, 48.67 \pm 9.32, 45.33 \pm 11.94, 48.67 \pm 7.19, 46.00 \pm 7.98, 38.00 \pm 8.93 Units/mg protein/min and 24.00 \pm 8.20, 45.33 \pm 2.46, 38.00 \pm 3.97, 24.67 \pm 5.97, 38.00 \pm 8.05, 30.67 \pm 6.25, 22.67 \pm 1.69 Units/mg protein/min in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively (Table 1).

Table 1: SOD activity in serum, liver and kidney of rats treated with PHEM at various doses and silymarin in paracetamol treated rats (n=6)

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
Serum SOD (U/mL)	8.67 \pm 0.85 ^{ab}	6.67 \pm 0.55 ^a	11.05 \pm 0.79 ^b	9.52 \pm 1.06 ^{ab}	11.62 \pm 0.64 ^b	10.10 \pm 1.01 ^b	11.81 \pm 0.58 ^b
Liver SOD Units/mg protein/min	44.00 \pm 10.48 ^a	44.00 \pm 4.62 ^a	48.67 \pm 9.32 ^a	45.33 \pm 11.94 ^a	48.67 \pm 7.19 ^a	46.00 \pm 7.98 ^a	38.00 \pm 8.93 ^a
Kidney SOD Units/mg protein/min	24.00 \pm 8.20 ^a	45.33 \pm 2.46 ^a	38.00 \pm 3.97 ^a	24.67 \pm 5.97 ^a	38.00 \pm 8.05 ^a	30.67 \pm 6.25 ^a	22.67 \pm 1.69 ^a

Values with different superscript in a raw were significantly different ($P < 0.05$)

Mean values of blood catalase activity were 2.93 \pm 0.12, 2.91 \pm 0.05, 2.55 \pm 0.08, 2.52 \pm 0.09, 2.47 \pm 0.15, 4.76 \pm 0.73 and 5.83 \pm 0.16 molar/min in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively. Mean values of liver and kidney catalase activity were 9.21 \pm 3.65, 11.60 \pm 2.71, 12.77 \pm 3.77, 10.52 \pm

1.41, 7.83 \pm 1.01, 11.27 \pm 1.30, 7.27 \pm 1.25 U/mg protein and 8.80 \pm 1.32, 7.37 \pm 1.34, 7.16 \pm 1.68, 6.37 \pm 1.59, 5.21 \pm 0.86, 3.80 \pm 0.56, 4.97 \pm 1.06 U/mg protein in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively (Table 2).

Table 2: Catalase activity in blood, liver and kidney of rats treated with PHEM at various doses and silymarin in paracetamol treated rats (n=6)

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
Blood catalase (molar/min)	2.93 ± 0.12 ^a	2.91 ± 0.05 ^a	2.55 ± 0.08 ^a	2.52 ± 0.09 ^a	2.47 ± 0.15 ^a	4.76 ± 0.73 ^b	5.83 ± 0.16 ^b
Liver catalase (U/mg protein)	9.21 ± 3.65 ^a	11.60 ± 2.71 ^a	12.77 ± 3.77 ^a	10.52 ± 1.41 ^a	7.83 ± 1.01 ^a	11.27 ± 1.30 ^a	7.27 ± 1.25 ^a
Kidney catalase (U/mg protein)	8.80 ± 1.32 ^a	7.37 ± 1.34 ^a	7.16 ± 1.68 ^a	6.37 ± 1.59 ^a	5.21 ± 0.86 ^a	3.80 ± 0.56 ^a	4.97 ± 1.06 ^a

Values with different superscript in a raw were significantly different ($P<0.05$)

Mean values of blood GSH were 7.96 ± 0.30 , 9.53 ± 0.64 , 8.99 ± 0.99 , 8.69 ± 0.82 , 10.76 ± 0.33 , 8.67 ± 0.54 and 8.67 ± 0.42 molar in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively. Mean values of liver and kidney GSH levels were 0.21 ± 0.02 , 0.15 ± 0.02 , 0.20 ± 0.08 , 0.24 ± 0.04 , $0.22 \pm$

0.04 , 0.37 ± 0.10 , 0.29 ± 0.05 $\mu\text{g}/\text{mg}$ of tissue and 0.20 ± 0.07 , 0.13 ± 0.01 , 0.13 ± 0.01 , 0.11 ± 0.00 , 0.13 ± 0.01 , 0.12 ± 0.00 , 0.11 ± 0.00 $\mu\text{g}/\text{mg}$ of tissue in rats of group C1, C2, C3, C4, T1, T2 and T3, respectively (Table 3).

Table 3: Level of GSH in serum, liver and kidney of rats treated with PHEM at various doses and silymarin in paracetamol treated rats (n=6)

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
Blood GSH (molar)	7.96 ± 0.30 ^a	9.53 ± 0.64 ^a	8.99 ± 0.99 ^a	8.69 ± 0.82 ^a	10.76 ± 0.33 ^a	8.67 ± 0.54 ^a	8.67 ± 0.42 ^a
Liver GSH ($\mu\text{g}/\text{mg}$ of tissue)	0.21 ± 0.02 ^a	0.15 ± 0.02 ^a	0.20 ± 0.08 ^a	0.24 ± 0.04 ^a	0.22 ± 0.04 ^a	0.37 ± 0.10 ^a	0.29 ± 0.05 ^a
Kidney GSH ($\mu\text{g}/\text{mg}$ of tissue)	0.20 ± 0.07 ^a	0.13 ± 0.01 ^a	0.13 ± 0.01 ^a	0.11 ± 0.00 ^a	0.13 ± 0.01 ^a	0.12 ± 0.00 ^a	0.11 ± 0.00 ^a

Values with different superscript in a raw were significantly different ($P<0.05$)

Three different mobile phases were used to detect flavonoids and phenolic compounds in all extracts with the use of rutin and quercetin as flavonoid standards and gallic acid as phenolic standard. Rf values of various flavonoids and phenolic compounds in extracts of plants used in the study are presented in Table 4. The Rf value of rutin, quercetin and gallic acid was 0.59, 0.75 and 0.59, respectively. Different colours in fluorescent light at 365 nm indicated presence of 3, 4, 1, 4, and 4 flavanoid compounds in *Allium cepa*, *Andrographis paniculata*, *Boerhaavia diffusa*, *Gymnosporia Montana* and *Luffa echinata*, respectively without detection of rutin in extracts when mobile phase of rutin was used (Figure 1). However, 3, 1, 2, and 3 flavanoid compounds were separated from *Allium cepa*, *Andrographis paniculata*,

, *Gymnosporia Montana* and *Luffa echinata*, respectively when mobile phase of quercetin was used (Figure 2). The extract of *Allium cepa* showed presence of abundant quercetin in it. Few different flavonoids were also observed in extracts of other plants which developed different colours up on derivatization. All extracts were also screen for the presence of gallic acid and other phenolic compounds in plant material used in the study. Gallic acid was not detected in all tested extracts. However, more phenolic compounds were observed in *Allium cepa* and *Andrographis paniculata*. Two phenolic compounds were observed in extracts of *Capparis deciduas*. However, *Gymnosporia Montana* and *Luffa echinata* showed presence of only single phenolic compound (Figure 3).

Table 4: Detection of flavonoids and phenolic compounds in extracts of plants used in the study

Rf values of flavanoids with use of rutin as standard							
1	2	3	4	5	6	7	8
0.59	0.97	0.96	0.98	-	0.94	0.98	0.59
-	0.81	0.84	-	-	0.66	0.81	-
-	0.58	0.74	-	-	0.56	0.70	-
-	-	5.20	-	-	0.38	0.63	-
Rf values of flavanoids with use of quercetin as standard							
1	2	3	4	5	6	7	8
0.75	0.87	0.29	-	-	0.15	0.86	0.75
-	0.82	-	-	-	0.09	0.79	-
-	0.75	-	-	-	-	0.60	-
Rf values of phenolic compounds with use of gallic acid as standard							
1	2	3	4	5	6	7	8
0.59	0.85	0.63	-	-	0.48	0.76	0.59
-	0.81	0.51	-	-	0.65	-	-
-	0.7	0.26	-	-	-	-	-
-	-	5.2	-	-	-	-	-

Column 1 and 8: Standard; Column 2: *Allium cepa*; Column 3: *Andrographis paniculata*, Column 4: *Boerhaavia diffusa*; Column 5: *Capparis deciduas*; Column 6: *Gymnosporia Montana*; Column 7: *Luffa echinata*

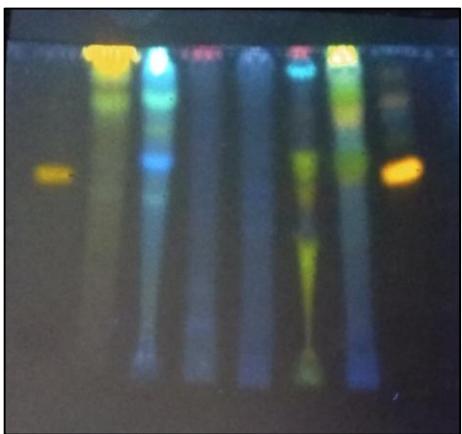


Fig 1: Detection of rutin and other flavonoids in extracts Orange colour indicates rutin. Row 1 and 8: Standard rutin; Row 2: *Allium cepa*; Row 3: *Andrographis paniculata*, Row 4: *Boerhaavia diffusa*; Row 5: *Capparis deciduas*; Row 6: *Gymnosporia Montana*; Row 7: *Luffa echinata*

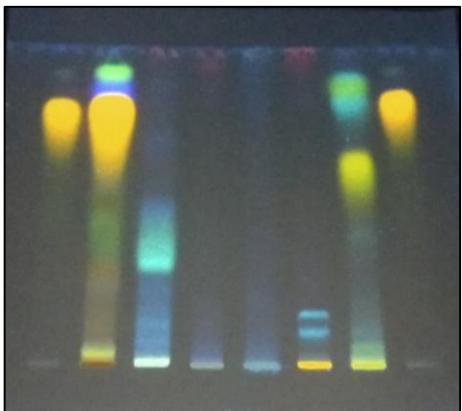


Fig 2: Detection of quercetin and other flavonoids in extracts Orange colour indicates quercetin. Row 1 and 8: Standard quercetin; Row 2: *Allium cepa*; Row 3: *Andrographis paniculata*, Row 4: *Boerhaavia diffusa*; Row 5: *Capparis deciduas*; Row 6: *Gymnosporia Montana*; Row 7: *Luffa echinata*

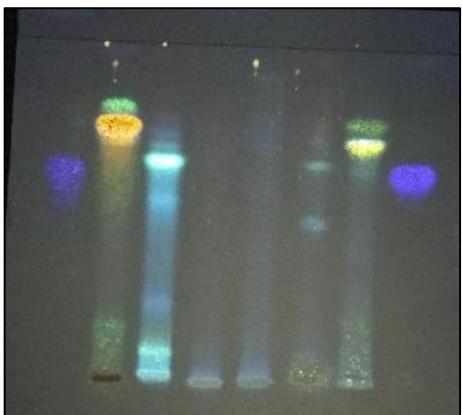


Fig 3: Detection of gallic acid and other phenolic compounds in extracts Purple colour indicates gallic acid. Row 1 and 8: Standard gallic acid; Row 2: *Allium cepa*; Row 3: *Andrographis paniculata*, Row 4: *Boerhaavia diffusa*; Row 5: *Capparis deciduas*; Row 6: *Gymnosporia Montana*; Row 7: *Luffa echinata*

4. Discussion

Superoxide dismutases (SODs) belong to a family of antioxidant enzymes that catalyze the dismutation of superoxide to yield hydrogen peroxide and oxygen. Two SODs are expressed intracellularly and present in the cytosol

in eukaryotic cells. In the present study, serum SOD activity was significantly increased in paracetamol treated rats. The activity of SOD in rats of silymarin and poly-herbal extract mixture groups (C4 and T2) were lower than mean value of SOD in rats treated with only paracetamol and were also comparable to the values observed in control rats. Liver and kidney SOD activities were non-significantly increased in paracetamol treated rats compared to other groups. It may due to more production of superoxide ion leads to elevation in SOD activity to overcome the auto oxidation and oxidative stress. If the oxidative stress is not very strong for long duration, the SOD activity increases. If oxidative stress is persisting or its level very high, the proteins damage became profound and a decreased SOD activity may occur either via direct oxidative damage of the SOD molecules, or via oxidative stress-altered SOD gene expression, or both. Treatment with chlorfenvinphos at low dose could result in an increase in the superoxide anion level and simultaneous increase in SOD activity [13]. The activity of SOD in liver and kidney of rats treated with higher dose (T3) of PHEM were non-significantly lower as compared to paracetamol treated rats.

Catalase is a ubiquitous enzyme which is present in the peroxisomes that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production [14]. In toxicity, the blood catalase is utilized more due to production of more ROS and H₂O₂. In the present study, blood catalase activity was non-significantly decrease in paracetamol treated rats but the same was significantly higher in rats treated with PHEM at 200 and 300 mg/kg (T2 and T3) as compared to value in rats treated with only paracetamol. However, we assumed that the increase in the activity of SOD in serum was not sufficient to detoxify the excess of superoxide anion. This could lead to a decrease in catalase activity in the experiment. Similar observation was also reported in rats exposed to chlorfenvinphos [15]. Catalase is inducible enzyme in tissues like liver and kidney. In the toxicity condition, there is more accumulation of drugs in this organs leads to more metabolite production which increase more ROS and oxidative stress. In the present study, liver catalase activity was non-significantly increase in paracetamol treated rats but in kidney there was no elevation of catalase activity was observed due to less toxic effect of paracetamol on kidney in the study. The increased activity of SOD was accompanied by the increase in the activity of catalase. The increase in hepatic catalase activity can be explained as the response of liver to high levels of H₂O₂. However, low activity of catalase in kidney indicates low level of toxicity of the drug in the study which might be due to less production of H₂O₂ in kidney.

GSH is an intra-cellular reluctant and plays a major role in catalysis, metabolism, and transport. It protects cells against free radicals, peroxides, and other toxic compounds [16]. In the present study, no alteration was observed in the GSH level in the blood and kidney. However, the liver GSH level in rats treated with silymarin and poly-herbal mixture (200 and 300 mg/kg) were higher than those values observed in other groups. Alteration in all above oxidative parameters may depend on dose and duration of drug or toxic substances.

5. Conclusions

Paracetamol administration at the dose rate of 500 mg/kg produced mild and continuous oxidative alterations in rats

which resulted in alterations in SOD and catalase in blood, liver and kidney tissues. Simultaneous administration of PHEM and silymarin produced reversal or attenuated effect on oxidative alterations caused by paracetamol in rats. This antioxidant effect of PHEM was due to presence of various flavonoids and phenolic compounds in extracts of plants used in the study. Further, it is needed to evaluate an antioxidant potential of the PHEM against high dose of oxidative stress inducer.

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