Original Article

Hepatoprotective Potential of *Tinospora cordifolia* and *Eclipta alba* Extract Compared with Market Formulation

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Doi : 10.47750/pnr.2022.13.S05.334

Abstract

In present research randomized open experimental study design was used, the study was conducted over the duration of 3years using 120 Rabbits. The animals were divided in 12 groups of 10 animals each. Oxidative stress was induced with Paracetamol. The sample size was calculated by using formula. All the drugs were administered orally as the schedule from immediately after Paracetamol administration, for 7 days continued. The blood sample were withdrawn from the marginal ear vein at 0,2,15,30,45,60 and 90 days. The single dose of all antioxidant under study including Liv-52 administered immediately after Paracetamol administration was found to be less effective as compared to once daily administration for seven days. On comparisons of the efficacy of antioxidants used in this study apparently *Tinospora cordifolia* was found to be most effective with respect to extent in decreasing MDA, ALT, AST levels and increasing SOD levels. *Tinospora cordifolia* also brought MDA, SOD, ALT and AST levels to the control values on 45 days. Liv-52 produced more or less same results. *Eclipta alba*, Vitamin C reached the control values on 60 days.

Key words: Tinospora cordifolia, Paracetamol, Eclipta alba, Vitamin C, Liv-52

INTRODUCTION

The use of plants as medications date back to the Renaissance and the accumulated traditional knowledge gained over the years has led to the creation of several innovative pharmaceuticals derived from plant-based biologically active components. Even today, the great majority of people in the globe still rely on native medicinal plants and products produced from plants for their daily health and care requirements [1-3]. A significant portion of the population in developing countries uses traditional medicines, as Cunningham (1988) correctly noted. This may be due to the high cost of western pharmaceuticals and health care, as well as the fact that traditional medicines are more acceptable from a cultural and spiritual stand point [4]. According to data from the WHO, traditional medicine is the primary source of treatment for 70% to 95% of individuals in the majority of under developed nations (Robinson and Zhang, i2011). Herbal medications are becoming a significant part of not just conventional medical and health care systems, but also the nutraceuticals and cosmetics industries. One-fourth of all medical prescriptions are based on formulations using chemicals that are derived from plants or their synthetic counter parts. Above all, it is thought that medications made from medicinal plants have the extra benefit of being straight forward, efficient, and providing a wide range of activities with a focus on the preventative action [5-8]. There are more than 120 different chemical compounds originating from plants that are used as major medications in one or more nations throughout the world right now.

Many of the medications were either replicas of naturally occurring compounds, processed plant components, or produced through straight forward synthetic changes. Natural medications are gaining popularity in the current global environment because they are affordable, accessible, and largely free from side effects. Traditional medical practices are seen as an intrinsic component of the culture in the majority of developing nations [9-13]. Three-fourths of the world's population, who are unable to buy contemporary synthetic treatments, is forced to rely on the usage of traditional medicines, which are mostly produced from plants [14-16].

MATERIAL AND METHOD

Animals

Wistar rats of either sex male or female weighing 200-220gm was used. The animals had access to food and water *add libitum*. The animals were housed in clean standard polypropylene cages in the laboratory animal room and maintained under standard conditions of temperature and humidity with an alternating 12-hour light and dark cycle. Other conditions of maintenance were kept in agreement with the guidelines set by B.R NAHATA COLLEGE OF PHARMACY NEEMUCH ROAD, MANDSAUR M.P, PHARMACY COUNCIL OF INDIA.

Collection of plant material

The whole plant of *Tinospora cordifolia and Eclipta alba* was collected from Apharwat (Gulmarg), M.P State, in the month of September. All the samples were acknowledged and validated by the Centre of Plant Taxonomy, Department of Botany, SAIFIA SCIENCE COLLEGE BHOPAL M.P . A reference specimen has been preserved in the herbarium of the Department of Botany, SAIFIA SCIENCE COLLEGE BHOPAL M.P under the voucher specimen number 2211-KASH.

Extract preparation

The collected plant material was cleaned and separated in to root, stem and leaf. All the parts were then shade dried at room temperature.The completely dried material was ground in powder using a grinder. The powder was extracted with different solvents using soxhlet extractor, filtered and concentrated with rotary evaporator. The temperature of soxhlet was set according to the boiling temperature of respective solvents. The solvent free extracts of root, stem and leaf were then stored at 4ºC till further use.

Qualitative phytochemical screening

Qualitative analysis of plant extracts was carried out using standard procedures to identify the major phytochemical constituents [17, 18]. These methods are as follows:

Test for alkaloids

A weighted amount of 0.5g of each of the plant extract was diluted to 10ml with acid alcohol. The mixture was then boiled and filtered. To filtrate was added 2ml of dilute ammonia. It was followed by addition of equal volume of chloroform to extract the alkaloidal base. Then chloroform layer was extracted using 10ml of acetic acid. This was divided into two portions. The upper layer was removed and to remaining part Draggendorff's reagent was added. The formation of reddish-brown precipitate with indicates the presence of alkaloids.

Test for terpenoids (Salkowski Test)

An amount equal to 0.5g of each extract was mixed in 2 ml of chloroform, and concentrated H2SO4 (2ml) was added cautiously to form a layer. Appearance of reddish brown colour at interface indicated the presence of terpenoids.

Test for saponins

The test extracts were vigorously shaken with distilled water and then allowed to stand for 5 minutes. Presence of stable froth more than 2 cm indicated the presence of saponins.

Test for flavonoids

Dilute ammonia solution (5ml) was added to a 2ml of the aqueous filtrate of plant extract to be tested. This was followed by addition of concentrated H_2SO_4. Appearance of yellow colour in the solution was regarded as presence of flavonoids

Test for steroids

An amount equal to 0.6g of plant extract was taken and 2ml of acetic acid was added to it After that 2ml H2SO4 was added to the solution The appearance of blue or green indicates the presence of steroids.

Test for tannins

About 0.6g of each of the plant extract was boiled in 6ml of water. The solution wasfiltered and 1-2drops of ferric chloride (0.1) were added to it. Appearance of brownish -green colouration indicates the presence of tannins.

Test for anthraquinones

Half a gram of plant extract was boiled with 5ml chloroform and then filtered. To the filtrate was added an equal volume of 10% ammonia solution. The mixture was then gently shaken. Appearance of pink colour on upper aqueous layer indicates the presence of anthraquinones.

Test for cardiac glycosides (Keller-Killiani Test)

An amount equal to 0.5g of plant extract was taken and 4ml of distilled water was added to it. Then to this mixture, 2ml glacial acetic acid and a drop of 2.0% FeCl3 mixture was added. A brown ring if formed between the layers indicates the presence of cardiac glycosides.

Test for phenols (Ferric chloride test)

A small quantity of plant extract was dissolved in 2ml of distilled water, and a 1-2 drops of ferric chloride solution (10%) were added to it. Appearance of blue-green colour produced indicates the presence of phenols.

RESULTS AND DISCUSSION

In *T. cordifolia*, carbohydrates were found only in the ethyl acetate extract of the seed while coumarins and tannins showed positive only in the methanol extract. Among ethanol, methanol, chloroform and ethyl acetate extracts; saponins and quinones were present only in ethanol extract of *E. alba* whereas phenols and tannins were not expressed in all four solvent

extracts. Alkaloids, carbohydrates, with saponins occurred only in the methanol extract. Carbohydrates, saponins, flavonoids, phenols, tannins, amino acids, proteins while ethanol alone tested positive for oxalates. Amino acids and proteins were absent in all the seed extracts [19-21].

Table 1: Preliminary phytochemical evaluation of *Tinospora cordifolia* seed extracts.

S. NO.	Phytochemical Tests	Ethanol extract	Methanol extract	Ethyl acetate extract	Chloroform extract
1) Tests for Alkaloids					
a)	*Mayer's test*	+	+	-	-
b)	*Wagner's test*	+	+	-	-
c)	*Dragendroff's test*	+	+	-	-
2) Tests for Carbohydrates					
a)	*Molisch's test*	-	-	+	-
b)	*Fehling's test*:	-	-	+	-
3) Tests for Saponins					
a)	*Frothing test*	+	+	-	-
4) Tests for Flavanoids					
a)	*Shinoda's Test*	-	+	-	-
b)	*Sodium hydroxide test*	-	+	-	-
5) Tests for Phenols/ Tanins					
a)	*Ferric chloride test*	+	+	-	-
b)	*Iodine test*	-	+	-	-
6) Tests for Cardiac glycosides					
a)	*Keller kelliani test*	+	+	+	+
7) Tests for Amino acids & Proteins					
a)	*Ninhydrin Test*	-	-	-	-
8) Tests for Steroids/Terpenoids					
a)	*Libermann Burchard's test*	+	+	+	+
b)	Salkowski's Test	+	+	+	+
9) Tests for Quinones		+	+	-	-
10) Tests for Oxalates		+	-	-	-
11) Tests for Coumarins		-	+	-	-

*Note: (+) Positive test, (-) Negative test

Table 2: Preliminary phytochemical evaluation of *Eclipta alba* seed extracts

S. NO.	Phytochemical tests	Ethanol extract	Methanol extract	Ethyl acetate extract	Chloroform extract
1) Tests for Alkaloids					
a)	*Mayer's test*	+	+	-	-
b)	*Wagner's test*	+	+	-	-
c)	*Dragendroff's test*	+	+	-	-
2) Tests for Carbohydrates					
a)	*Molisch's test*	-	+	+	+
b)	*Fehling's test*:	-	+	+	+
3) Tests for Saponins					
a)	*Frothing test*	+	-	-	-
4) Tests for Flavanoids					
a)	*Shinoda's Test*	+	+	-	-
b)	*Sodium hydroxide test*	+	+	-	-
5) Tests for Phenols/ Tanins					
a)	*Ferric chloride test*	-	-	-	-
b)	*Iodine test*	-	-	-	-
6) Tests for Cardiac glycosides					
a)	*Keller kelliani test*	+	+	+	+
7) Tests for Amino acids & Proteins					
a)	*Ninhydrin Test*	-	-	-	-
8) Tests for Steroids/Terpenoids					
a)	*Libermann Burchard's test*	+	+	+	+
b)	Salkowski's Test	+	+	+	+
9) Tests for Quinones		+	-	-	-
10) Tests for Oxalates		-	-	-	-
11) Tests for Coumarins		+	+	-	-

*Note: (+) Positive test, (-) Negative test

Table 3: *Tinospora cordifolia* on MDA

Groups ↓	Time interval→							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	2.27±0.62	2.51±0.90	2.28±0.73	2.49±0.87	2.38±0.56	2.62±0.89	2.36±0.57	2.58±0.76
B	2.17± 0.40	10.69±0.43	10.73±0.77	10.19±0.67	10.34±0.79	10.25±0.14	10.87±0.67	8.28±0.11
C1	2.70±0.57**	6.03±0.58**	4.47±0.71**	3.19±0.60**	2.46±0.63**	2.54±0.69**	2.36±0.76**	2.17±0.69**
D1	2.48±0.56*	6.23±0.23*	6.35±0.38*	5.89±0.91*	4.38±0.86*	3.82±0.08*	2.76±0.8*	2.34±0.09*

A= Control no drug. B= Paracetamol alone. C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days. D_1= Paracetamol Tinospora cordifolia single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 4: *Tinospora cordifolia* on SOD

Group s ↓	**Time interval →**							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	0.116±0.018	0.116±0.017	0.115±0.013	0.115±0.019	0.116±0.017	0.116±0.012	0.115±0.016	0.116±0.013
B	0.116±0.017	0.063±0.007	0.064±0.008	0.064±0.009	0.063±0.010	0.064±0.011	0.077±0.012	0.085±0.013
C_1	0.116±0.017**	0.076±0.010**	0.097±0.011**	0.109±0.012**	0.116±0.013**	0.116±0.014**	0.116±0.015**	0.116±0.016**
D_1	0.115±0.018*	0.078±0.010*	0.088±0.010*	0.097±0.010*	0.102±0.012*	0.107±0.012*	0.116±0.017*	0.116±0.014*

A= Control no drug. B= Paracetamol alone.
C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days.
D_1= Paracetamol+Tinospora cordifolia single dose immediately after Paracetamol administration.
**P<0.001 in comparison with Paracetamol control.
*P<0.05 in comparison with immediately.

Table 5: *Tinospora cordifolia* on Protective Index

Group ps ↓	Time interval →							
	Day 0	Day 2	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
A	0.054±0.010	0.055±0.010	0.056±0.011	0.054±0.011	0.054±0.010	0.054±0.010	0.055±0.011	0.054±0.011
B	0.053±0.012	0.006±0.002	0.006±0.001	0.007±0.001	0.007±0.001	0.009±0.002	0.016±0.002	0.021±0.005
C1	0.053±0.009**	0.024±0.009**	0.036±0.012**	0.041±0.008**	0.054±0.013**	0.055±0.001**	0.054±0.007**	0.055±0.008**
D1	0.055±0.011*	0.026±0.003*	0.031±0.004*	0.035±0.003*	0.037±0.005*	0.042±0.007*	0.054.009*	0.054±0.008*

A= Control no drug. B= Paracetamol alone. C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days. D_1= Paracetamol+ Tinospora cordifolia single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 6: *Tinospora cordifolia* on Stress Index

Grou ps ↓	Time interval→							
	day0	Day2	Day15	Day30	day45	day60	day75	day90
A	22.06±1.23	22.45±1.28	20.34±1.74	22.25±1.09	21.2±1.45	22.27±1.70	21.03±1.60	22.58±1.99
B	22.76±1.08	91.1±8.33	119.72±17.34	122.31±7.26	125.21±4.13	129.81±13.49	118.83±10.39	109.16±11.23
C1	22.47±2.27	54.18±6.14	42.88±5.05	34.08±3.41	24.10±2.11	24.43±3.81	24.38±2.95	24.13±2.11
D1	22.83±2.15	56.83±7.25	52.39±7.97	46.48±5.53	37.86±5.01	29.50±8.34	24.42±1.66	23.17±2.47

A= Control no drug. B= Paracetamol alone. C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days. D_1= Paracetamol+ Tinospora cordifolia single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 7: *Tinospora cordifolia* on ALT

Grou ps ↓	Time interval→							
	day0	Day2	Day 15	Day30	day 45	day60	day75	day90
A	51.8±4.56	52.4±5.19	51.8±5.37	50.9±4.32	51.7±4.47	52.7±5.79	52.5±4.87	52.8±5.65
B	51.7±5.43	98.5±7.23	112.7±9.32	118.4±10.59	127.2±12.56	136.4±20.73	23.8±17.12	113.7±11.35
C_1	51.6±5.83**	82.5±9.57**	1.6±8.79**	3.2±7.13**	52.4±6.14**	51.3±6.99**	53.1±7.19**	52.9±6.45**
D_1	52.8±5.25	86.2±7.43	92.6±7.47	86.5±6.99	70.3±6.98	61.4±5.90	51.7±5.66	51.1±5.88

A= Control no drug. B= Paracetamol alone. C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days. D_1= Paracetamol+ Tinospora cordifolia single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

Table 8: *Tinospora cordifolia* on AST

Grou ps ↓	Time interval→							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	22.5±1.23	21.3±1.98	22.8±1.43	20.8±1.37	21.7±1.34	20.9±1.69	21.1±1.45	22.7±1.56
B	20.7±1.43	51.5±5.23	66.7±7.32	77.4±8.59	87.2±8.56	99.4±9.73	87.8±8.99	85.4±7.39
C_1	20.4±1.56**	39.5±5.00**	34.7±4.36**	29.4±2.67**	22.9±2.19**	21.7±2.44**	20.3±2.16**	22.3±2.19**
D_1	21.7±1.20	42.3±4.88	39.5±4.33	35.9±6.12	30.5±4.02	26.9±3.99	22.5±3.16	22.1±1.49

A= Control no drug. B= Paracetamol alone. C_1 = Paracetamol+ Tinospora cordifolia immediate and daily for 7 days. D_1= Paracetamol+Tinospora cordifolia single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

Table 9: *Eclipta alba* on MDA

Grou ps ↓	Time interval→							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	2.27±0.62	2.51±0.90	2.28±0.73	2.49±0.87	2.38±0.56	2.62±0.89	2.36±0.57	2.58±0.76
B	2.17±0.40	10.69±0.43	10.73±0.77	10.19±0.67	10.34±0.79	10.25±0.14	10.87±0.67	8.28±0.11
C_3	2.57±0.78**	6.19±0.71**	5.12±0.64**	4.17±0.92**	3.24±0.69**	2.56±0.75**	2.35±0.88**	2.19±0.85**
D_3	2.03±0.80*	6.48±0.37*	5.98±0.38*	4.91±0.27*	4.11±0.13*	3.84±0.12*	3.13±0.56*	2.49 ± 0.56*

A= Control no drug. B= Paracetamol alone. C_3= Paracetamol+ Eclipta alba immediate and daily for 7 days. D_3= Paracetamol+ *Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 10: *Eclipta alba* on SOD

Grou ps ↓	Time interval →							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	0.116±0.018	0.116±0.017	0.115±0.013	0.115±0.019	0.116±0.017	0.116±0.012	0.115±0.016	0.116±0.013
B	0.116±0.017	0.063±0.007	0.064±0.008	0.064±0.009	0.063±0.010	0.064±0.011	0.077±0.012	0.085±0.013
C_3	0.115±0.019**	0.082±0.013**	0.096±0.016**	0.103±0.017**	0.108±0.018**	0.116±0.015**	0.116±0.013**	0.116±0.011**
D_3	0.114±0.017*	0.080±0.022*	0.079±0.023*	0.086±0.024*	0.090±0.023*	0.098±0.016*	0.103±0.023*	0.116±0.018*

A= Control no drug. B= Paracetamol alone. C_3= Paracetamol+*Eclipta alba* immediate and daily for 7 days. D_3= Paracetamol+*Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 11: *Eclipta alba* on Protective Index

Grou ps ↓	Time interval→							
	day 0	Day 2	Day 15	Day 30	day 45	day 60	day 75	day 90
A	0.054±0.010	0.055±0.010	0.056±0.011	0.054±0.011	0.054±0.010	0.054±0.010	0.055±0.011	0.054±0.011
B	0.053±0.012	0.006±0.002	0.006±0.001	0.007±0.001	0.007±0.001	0.009±0.002	0.016±0.002	0.021±0.005
C_3	0.055±0.019**	0.028±0.010**	0.036±0.003**	0.040±0.005**	0.047±0.005**	0.054±0.003**	0.054±0.005**	0.054±0.012**
D_3	0.055±0.006*	0.027±0.010*	0.029±0.003*	0.031±0.003*	0.035±0.004*	0.043±0.006*	0.048±0.007*	0.054±0.006*

A= Control no drug. B= Paracetamol alone. C_3= Paracetamol+ *Eclipta alba* immediate and daily for 7 days. D_3= Paracetamol+ *Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 12: *Eclipta alba* on Stress Index

Grou ps ↓	Time interval→							
	day0	Day2	Day 15	Day30	day 45	day60	day75	day90
A	22.06±1.23	22.45±1.28	20.34±1.74	22.25±1.09	21.2±1.45	22.27±1.70	21.03±1.60	22.58±1.99
B	2.76±1.08	91.1±8.33	119.72±17.34	122.31±7.26	125.21±4.13	129.81±13.49	118.83±10.39	109.16±11.23
C_3	21.19±1.87**	73.75±9.94**	61.06±8.82**	51.06±7.30**	34.69±3.54**	22.55±3.26**	22.15±2.81**	22.33±1.78**
D_3	21.31±1.93*	75.57±10.03*	71.37±8.21*	62.45±5.63*	52.13±5.79*	43.33±3.99*	34.50±3.667*	22.38±2.23*

A= Control no drug. B= Paracetamol alone. C_3=Paracetamol+*Eclipta alba* immediate and daily for 7 days. D_3= Paracetamol+*Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 13: *Eclipta alba* on ALT

Grou ps ↓	Time interval→							
	day 0	day 2	day 15	day 30	day 45	day 60	day 75	day 90
A	51.8±4.56	52.4±5.19	51.8±5.37	50.9±4.32	51.7±4.47	52.7±5.79	52.5±4.87	51.8±5.65
B	51.7±5.43	98.5±7.23	112.7±9.32	118.4±10.59	127.2±12.56	136.4±20.73	123.8±17.12	113.7±11.35
C_3	51.4±5.83**	88.7±8.52**	74.6±7.75**	67.9±6.47**	61.5±6.54**	52.5±6.88**	52.4±7.12**	51.1±5.96**
D_3	52.8±5.25	89.3±9.23	86.4±9.45	79.8±8.55	70.4±7.38	64.2±6.94	52.9±6.35	51.1±6.45

A= Control no drug. B= Paracetamol alone. C_3 =Paracetamol+*Eclipta alba* immediate and daily for 7 days. D_3=

Paracetamol+*Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

Table 14: Eclipta alba on AST

Grou ps ↓	Time interval→							
	day 0	day 2	day 15	day 30	day 45	day 60	day 75	day 90
A	22.5±1.23	21.3±1.98	22.8±1.43	20.8±1.37	21.7±1.34	20.9±1.69	21.1±1.45	22.7±1.56
B	20.7±1.43	51.5±5.23	66.7±7.32	77.4±8.59	87.2±8.56	99.4±9.73	87.8±8.99	85.4±7.39
C_3	20.1±1.23**	48.6±3.29**	40.2±3.77**	34.9±2.35**	28.5±1.22**	22.7±1.28**	22.8±1.23**	22.5±1.73**
D_3	20.1±1.44	50.4±4.16	47.8±6.47	43.7±6.78	37.5±4.64	33.9±2.11	28.4±1.35	22.3±1.93

A= Control no drug. B= Paracetamol alone. C_3 = Paracetamol + *Eclipta alba* immediate and daily for 7 days. D_3= Paracetamol + *Eclipta alba* single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

Table 15: Vitamin C on MDA

Groups ↓	Time interval→							
	day 0	day 2	day 15	day 30	day 45	day 60	day 75	day 90
A	2.27±0.62	2.51±0.90	2.28±0.73	2.49±0.87	2.38±0.56	2.62±0.89	2.36±0.57	2.58±0.76
B	2.17±0.40	10.69±0.43	10.73±0.77	10.19±0.67	10.34±0.79	10.25±0.14	10.87±0.67	8.28±0.11
C_4	2.93±0.77**	6.04±0.87**	5.20±0.72**	4.57±0.69**	3.46±0.80**	2.61±0.70**	2.48±0.75**	2.25±0.69**
D_4	2.42±0.64*	6.09±0.72*	6.18±0.92*	5.34±0.83*	4.58±0.80*	3.79±0.94*	2.73±0.04*	2.45±0.64*

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4= Paracetamol+ Vitamin C single dose immediately after Paracetamol administration.
**P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 16: Vitamin C on SOD

Groups ↓	Time interval →							
	day0	day2	day15	day30	day45	day60	day75	day90
A	0.116±0.018	0.116±0.017	0.115±0.013	0.115±0.019	0.116±0.017	0.116±0.012	0.115±0.016	0.116±0.013
B	0.116±0.017	0.063±0.007	0.064±0.008	0.064±0.009	0.063±0.010	0.064±0.011	0.077±0.012	0.085±0.013
C_4	0.115±0.018**	0.081±0.011**	0.095±0.013**	0.105±0.010**	0.109±0.015**	0.116±0.014**	0.116±0.012**	0.116±0.015**
D_4	0.115±0.020*	0.083±0.011*	0.086±0.010*	0.094±0.012*	0.101±0.017*	0.107±0.013*	0.116±0.015*	0.116±0.016*

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4= Paracetamol+ Vitamin C single dose immediately after Paracetamol administration.
**P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 17: Vitamin C on Protective Index

Grou ps ↓	Time interval→							
	day0	day 2	day15	day30	day45	day60	day75	day90
A	0.054±0.010	0.055±0.010	0.056±0.011	0.054±0.011	0.054±0.010	0.054±0.010	0.055±0.011	0.054±0.011
B	0.053±0.012	0.006±0.002	0.006±0.001	0.007±0.001	0.007±0.001	0.009±0.002	0.016±0.002	0.021±0.005
C_4	0.055±0.011**	0.031±0.0012**	0.039±0.011**	0.044±0.012**	0.047±0.011**	0.054±0.014**	0.054±0.016**	0.054±0.015**
D_4	0.056±0.0013*	0.032±0.008*	0.035±0.009*	0.038±0.0013*	0.042±0.009*	0.047±0.012*	0.054±0.012*	0.054±0.011*

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4=Paracetamol +Vitamin C single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 18: Vitamin C on stress index

Grou ps ↓	Time interval→							
	day 0	day2	day15	day30	day45	day60	day75	day90
A	22.06±1.23	22.45±1.28	20.34±1.74	22.25±1.09	21.2±1.45	22.27±1.70	21.03±1.60	22.58±1.99
B	22.76±1.08	91.1±8.33	119.72±17.34	122.31±7.26	125.21±4.13	129.81±13.49	118.83±10.39	109.16±11.23
C_4	22.47±1.89**	47.25±3.55**	35.52±3.45**	32.87±2.53**	29.29±2.43**	22.73±2.18**	22.59±1.32**	22.26±1.11**
D_4	20.77±1.14*	48.11±6.05*	44.96±5.52*	39.76±4.45*	34.58±3.75*	30.23±2.87*	22.47±2.73*	22.28±2.23*

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4=Paracetamol +Vitamin C single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control. *P<0.05 in comparison with immediately.

Table 19: Vitamin C on ALT

Grou ps	Time interval→							
↓	day 0	day 2	day 15	day 30	day 45	day 60	day 75	day 90
A	51.8±4.56	52.4±5.19	51.8±5.37	50.9±4.32	51.7±4.47	52.7±5.79	52.5±4.87	52.8±5.65
B	51.7±5.43	98.5±7.23	112.7±9.32	118.4±10.59	127.2±12.56	136.4±20.73	123.8±17.12	113.7±11.35
C_4	51.9±5.83**	76.7±9.38**	71.5±8.49**	66.1±7.16**	62.6±6.55**	51.7±6.21**	51.9±6.39**	52.4±5.99**
D_4	52.8±5.25	80.3±8.65	80.2±9.12	75.7±9.19	70.4±7.01	64.1±7.76	51.7±6.17	51.4±6.73

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4= Paracetamol+ Vitamin C single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

Table 20: Vitamin C on AST

Grou ps	Time interval→							
↓	day0	day2	day15	day30	day45	day60	day75	day90
A	22.5±1.23	21.3±1.98	22.8±1.43	20.8±1.37	21.7±1.34	20.9±1.69	21.1±1.45	22.7±1.56
B	0.7±1.43	51.5±5.23	66.7±7.32	77.4±8.59	87.2±8.56	99.4±9.73	87.8±8.99	85.4±7.39
C_4	20.5±1.24**	46.3±5.37**	41.5±3.66**	37.8±2.05**	30.5±2.29**	22.7±1.33**	22.4±1.17**	22.3±1.84**
D_4	23.4±1.97	47.5±5.24	42.1±6.16	38.7±6.11	33.1±5.09	26.9±2.59	22.5±1.66	22.4±1.38

A= Control no drug. B= Paracetamol alone. C_4 = Paracetamol+ Vitamin C immediate and daily for 7 days. D_4= Paracetamol+ Vitamin C single dose immediately after Paracetamol administration. **P<0.001 in comparison with Paracetamol control.

DISCUSSION

The present study was undertaken to compare antioxidant and hepatoprotective activity of the plants *Tinospora cordifolia*, *Eclipta alba* comparing with Vitamin C and Liv-52. The indicators used to evaluate antioxidant activity are MDA, SOD, Protective index and Stress index. Hepatoprotective activity was quantified using ALT and AST as liver function test parameter. These treatments were also compared with respect to the on cost comparison of various drugs under present study. Further, Stress index, Protective index and corresponding ALT and AST values were examined for co-relation by using the method of correlation coefficient. In present research randomized open experimental study design was used. The study was conducted over the duration of 3 years using 120 Rabbits. The animals were divided in 12 groups of 10 animals each. Oxidative Stress was induced with Paracetamol. The sample size was calculated by using formula. All the drugs were administered orally as the following schedule from immediately after Paracetamol administration, immediately after Paracetamol dosing and administered each day for 7 days continued. The blood sample were withdrawn from the marginal ear vein at day 0, day 2, day15, day 30, day 45, day 60, days75 and day 90. The single dose of all antioxidant under study including Liv-52 administered immediately after Paracetamol administration was found to be less effective as compared to once daily administration for seven days. On comparisons of the efficacy of antioxidants used in this study apparently *Tinospora cordifolia* was found to be most effective with respect to extent in decreasing MDA, ALT, AST levels and increasing SOD levels. *Tinospora cordifolia* also brought MDA, SOD, ALT, and AST levels to the control values on 45 days. Liv-52 produced more or less same results. Eclipta alba, Vitamin C reached the control values on 60 days. However on application of the tests of significant to the differences of efficacy of antioxidant under study the level of significance could not be demonstrated. This could be either due to a comparatively smaller size or nature of the animal species used for these enzymatic studies. Further studies are needed for demonstration of statistically significant rank order of efficacies of these antioxidants.

CO-RELATION COEFFICIENT BETWEEN STRESS INDEX, PROTECTIVE INDEX AND ALT, AST

a) Correlation between Stress index and ALT

On comparison of values of Stress index and corresponding ALT values for calculation of correlation coefficient it was observed to be 0.9007 which lies close to 1. This suggests that there is a strong correlation between values of Stress index and ALT hence the values of Stress index can be predicted from the known values of ALT and vice versa after plotting the standard curve.

b) Correlation between stress index and AST

On comparison of the values of Stress index and corresponding values of AST for calculation of correlation coefficient the correlation coefficient was observed to the to be 0.8340 which lies close to 1. This suggests that there is strong correlation between values of Stress index and AST hence the values of Stress index can be predicted from the known values of AST and vice versa after plotting the standard curve.

c) Correlation between protective index and ALT

When all the values of Protective index and corresponding values of ALT were plotted for calculation of correlation coefficient the correlation coefficient was observed to the to be 0.9193 which lies close to 1. This suggests that there is strong correlation between values of Protective index and ALT. Hence the values of Protective index can be predicted from the known values of ALT and vice versa after plotting the standards curve.

d) Correlation between protective index and AST:

When all the values of Protective index and corresponding values of AST for correlation coefficient were compared the correlation coefficient was observed to be 0.924 which lies close to1. This suggests that there is a strong correlation between values of Protective index and AST. Hence the values of Protective index can be predicted from the known values of AST and vice versa after plotting the standards curve.

On cost comparison of various drug under present study *Tinospora cordifolia* which is as effective asLiv-52 pulp was found to be the cheapest drug having both antioxidant and hepatoprotective activity followed by Eclipta alba pulp, Vitamin c tab, Liv-52 syrup.

CONCLUSION

Hepatoprotective activity of *Tinospora cordifolia*, *Eclipta alba*, Vitamin C and Liv52 is maximum when each drug is given immediately after producing hepatotoxicity and continue for seven days after producing hepatotoxicity. There is no significant difference between their activities but. In *Tinospora cordifolia* treated group quick results. There is no significant difference between these activities but the quick action was shown by *Tinospora cordifolia*. In our study we found that *Tinospora cordifolia* which is as effective as Liv-52, was found to be the most cost effective treatment for Paracetamol induced hepatotoxicity followed by *Tinospora cordifolia*, Eclipta alba, Vitamin C and Liv-52. A strong Co-correlation exists between values of ALT, AST and Protective index, Stress index and values of MDA SOD with the correlation coefficients 0.9007, 0.8340, 0.9193, 0.9241 respectively. This strong correlation indicates that irrespective of the causative agent of hepatocellular damage final step is through excessive generation of reactive oxygen species (ROS). In the view of the above it is prudent to prescribe appropriate antioxidant in appropriate doses as a matter of routine whenever hepatotoxic or potentially hepatotoxic drugs are prescribed and also as a preventive measure everyone should take diet rich in antioxidants.

REFERENCE

1. Sujithra R. Chyawanprash from Vedic to Genomic Era. Ancient Science of Life. 2013; 33(1):76-.
2. Irshad M, Chaudhuri PS. Oxidant-Antioxidant System: Role and Significance in Human Body. Indian Journal of Experimental Biology. 2022; 40: 1233-1239.
3. Kumar V, Abbas AK, Fausto N. Cellular Adaptations, Cell Injury and Cell Death. In: Robbins and Cotran, Eds., Pathologic Basis of Disease, Saunders, Philadelphia. 2009 15-18.
4. Bhuiyan MA, Hoque MZ, Hossain SJ. Free radical scavenging activities of Zizyphus mauritiana. World journal of agricultural sciences. 2009; 5(3):318-22.
5. Ramadoss S, Kannan K, Balamurugan K, Jeganathan NS, Manavalan R. Evaluation of hepato-protective activity in the ethanolic extract of Sida rhombifolia Linn. against paracetamol-induced hepatic injury in albino rats. RJPBCS. 2012; 3:497-501.
6. Kumar KS, Kumar KS. Hepatoprotective effects of 50% ethanolic extract of Ficus hispida Linn against CCl4 induced hepatotoxicity in rats. European Journal of Biological Sciences. 2012; 4(1):01-4.
7. Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pradhan SC. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian Journal of Medical Research. 2009 May 1; 129(5):569.
8. Karole S, Jain DK, Abbas S, Abbas N, Kare P. Antioxidant and hepatoprotective effects of ougeinia dalbergioides benth against paracetamol and ccl4 induced liver damage in rats. Indian Journal of Pharmacy and Pharmacology. 2022; 9(1):67–74.
9. Ray G, Husain SA. Oxidants, antioxidants and carcinogenesis. Indian Journal of Experimental Biology.2002; 40 (9):1213-1232.
10. Rodrigo R, Guichard C, Charles R. Clinical pharmacology and therapeutic use of antioxidant vitamins. Fundamental & clinical pharmacology. 2007; 21(2):111-27.
11. Malik KK, Pal MB. Role of Liv. 52 in hepatitis and cirrhosis of the liver. Current Medical Practice. 1979; 5:23.
12. Jat D, Thakur N, Jain DK, Prasad S, Yadav R. Iris ensata Thunb: Review on Its Chemistry, Morphology, Ethno Medical Uses, Phytochemistry and Pharmacological Activities. Asian Journal of Dental and Health Sciences. 2022; 2(1):1-6.
13. Jena J, Gupta AK. Ricinus communis Linn: a phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(4):25-9.
14. Dalal S, Kataria SK, Sastry KV, Rana SV. Phytochemical screening of methanolic extract and antibacterial activity of active principles of hepatoprotective herb, Eclipta alba. Ethnobotanical leaflets. 2010; (3):3.
15. Globale PH. Hepatoprotective Activity of Ethanolic Extract of Stachytarphetaindica on Wistar Rats.2011; 1(1):1-3.
16. Lee WM. Drug-induced hepatotoxicity. New England journal of medicine. 2003; 349(5):474-85.
17. Parkhe G, Jain P, Jain DK. Total phenolic content, total flavonoid & anti-bacterial activity of hydroalcoholic acacia catechu (l.f.) Willd roots extracts for treatment of mouth ulcer. Pharmacologyonline 2018; 2: 227-233.
18. Patel NS, Jain DK, Nagar H, Patel A, Chandel HS. Evaluation of analgesic and antipyretic activity of Tridax procumbens leaves extract. RGUHS J Pharm Sci. 2011; 1(3):226-31.
19. Patel NS, Jain DK, Nagar H, Patel A, Chandel HS. Evaluation of analgesic and antipyretic activity of Tridax procumbens leaves extract. RGUHS J Pharm Sci. 2011; 1(3):226-31.

20. Pathak AK, Jain DK, Gupta S, Raguwansi MB, Chandel HS. Wound healing activity of polyherbal formulation on excision based wound model in mice. Plant Archives 2008; 8(2):783-785.
21. Nagar H, Tiwari P, Jain DK, Chandel HS. Evaluation of Anti-ulcer activity of stem bark extract of Aphanmixis polystachya in experimental rats. Ind J Pharm Edu Res. 2012; 46(3):222-7.