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ACTILIFE TABLET AND GREEN TEA EXTRACT ACT SYNERGISTICALLY TO PROTECT RAT LIVER AGAINST PARACETAMOL TOXICITY

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Abstract

In the present study protective effect of Actilife tablets (AT) and Green tea extract (GTE) against paracetamol induced hepatotoxicity was evaluated individually and in combination. Rats are pretreated with AT (50 and 100 mg/kg, p.o), GTE (5 and 10 mg/kg, p.o), and combination formulation, ATG (40 and 80 mg/kg, p.o), for 14 days. On day 14 paracetamol was administered (2.5 g/kg, p.o) and after 48 h serum was analyzed for ASAT, ALAT, ALP and TP. The liver tissue was analyzed for SOD, catalase, GSH, GPx, MDA and histopathology. AT, GTE and ATG show a dose dependent protection against the paracetamol induced changes in serum and liver tissue parameters($p<0.05$). When compared to AT and GTE, the combination formulation, ATG, shows a superior hepatoprotection even at doses below their individual equivalent doses. The results, therefore indicate synergism between the ingredients of AT and GTE.

Keywords: Hepatoprotective, Synergistic effect, Actilife tablet, Green tea extract, Paracetamol, Hepatotoxicity.

Introduction

Liver disease is recognized as the second leading cause of mortality amongst all gastrointestinal disease and the rates are steadily increasing over the years(1). According to National statistics in the UK, liver diseases have been ranked as the fifth most common cause of death and globally more than 1300 million people are affected(2). Attention has been paid to the protective effects of natural antioxidants against xenobiotics induced toxicities especially whenever free radicals are involved. Natural drugs are therefore, one of the major contributors in the treatment of liver disorders (3).Currently, a handful of polyherbal formulations, namely Silymarin, Liv- 52, Hepatomed, Livfit, Stimuliv, Himoliv, Tefroliv,etc, have been scientifically validated(4, 5). However, there are many formulations still need to be validated scientifically.

Actilife tablets (AT) is a multi-vitamin and multi-mineral formulation manufactured by M/s. Apex Laboratories, Chennai, India(Table-1). The formulation has been recommended as a dietary supplement to promote appetite, convalescence, growth and to treat vitamin and mineral deficiency. Green tea extract (GTE) has been reported to contain antioxidant phytoconstituents such as epigallocatechingallate, catechin, gallocatechin, epicatechinand caffeine. The various reports suggest the hepatoprotective activity of green tea extracts to their phytoconstituents (6). In the present study both AT and GTE are evaluated individually and in combination (ATG) for their hepatoprotective activity against paracetamol induced liver damage.

Materials and Methods

Chemicals

The standardized GTE (80% Polyphenols), AT, ATG were supplied by from M/s. Apex Laboratories, Chennai, India. The Serum aspartate aminotransferase (ASAT), Alanine aminotransferase(ALAT), Alkaline phosphatase (ALP) and Total Proteins (TP) Kits were from Precision Biomed (P) Ltd, Jaipur, India. Reduced Glutathione(GSH), Superoxide dismutase(SOD), Catalase and Glutathione peroxidase(GPx) kits were from Cayman chemical company, Michigan, USA. Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ethylenediamine tetra acetic acid (EDTA), were purchased from Sigma-aldrich chemical co., Mumbai, India. All other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats (170-210g) were procured from in-house animal facility of J.S.S College of Pharmacy, Ootacamund. The animals were housed under standard conditions of temperature (22±3°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (M/s. Amrit feeds Ltd, Banglore, India) and water ad libitum. The experiment was conducted with prior approval of Institutional Animal Ethical Committee of JSS College of Pharmacy, Ooty, India (Approval No. JSSCP/IAEC/CADRAT/06/2014-15).

Acute oral toxicity studies

Acute oral toxicity of AT, GTE and ATG was carried out as per the OECD guideline for testing of the chemicals (7). A limit test at a dose of 2000 mg/kg, p.o., was carried out using 3 female rats per step. After administration of test samples, all the animals were observed for clinical signs and mortality at 0, 0.5, 1, 2 and 4 h, and thereafter daily for a period of 14 days. During the study period, weekly body weights of all the animals were recorded. At the end of the study, all the survived animals were culled by deep ether anaesthesia and subjected to gross necropsy analysis.

Hepatoprotective activity evaluation

Dose selection

Body surface area conversion factor was used to convert the human dose of AT, GTE and ATG to rat dose (Table-2).

Test item Preparation

All the test items were prepared as suspension in 0.5% CMC at a concentration equal to 1/10th of their dose and administered at a dose volume of 10ml/kg, b.wt. The test items were prepared immediately prior to administration and the homogenicity was maintained by constant stirring during administration.

Procedure

Animals were divided in to 8 groups of 6 each. Group 1 and 2 received vehicle (0.5% CMC, 10 ml/kg, p.o.) and served as normal and control, respectively. Group 3 &4 received AT at50 and 100mg/kg, p.o., respectively. Group 5 & 6 received GTE at 5 and 10mg/kg, p.o., respectively. Group 7 & 8 received ATG at 40 and 80 mg/kg, p.o., respectively. All the animals received the assigned treatment for a period of 14 days. On day 14 all the groups except Group-1 (Normal) received paracetamol suspension (2.5g/kg, p.o). After 48 h the blood (5ml) was collected from retro-orbital plexus under light ether anaesthesia for biochemical estimations. The animals were then sacrificed and liver was isolated and processed for estimation of antioxidant parameters and histopathology analysis.

Serum biochemical estimation

At the end of the study blood sample were collected from the retro-orbital plexus and allowed to clot for 45 min at room temperature. The serum was separated by centrifugation at 3000rpm at 30°c for 15 min and used for assaying ASAT, ALAT, ALP and TP using assay kits.

Tissue biochemical estimations

The animals were sacrificed by overdose of ether and liver was excised, washed with ice cold saline and weighed. A portion of the tissue was used for histopathology and remaining for the preparation of 10%w/v homogenate in ice-cold phosphate buffer (100mM, pH7.4). The homogenates were centrifuged at 10,000×g for 20 min and used for the estimation of malondialdehyde (MDA). GSH, SOD, catalase and GPx using assay kits.

Histopathological Studies

The liver tissue was fixed in the 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin wax. The sections which are 5-6 µm thick, were then prepared using rotary microtome and stained with hematoxylin and eosin dye for microscopic observation.

Statistical analysis

The data were presented as mean ±SD and analyzed by one-way ANOVA followed by Dunnett's multiple comparision tests using Prism software (Version 5.0) p-value ≤ 0.05 were considered significant.

Results

Acute oral toxicity studies

No abnormal clinical signs, mortality and body weight change were observed during the study period for all the animals treated with AT, GTE and ATG at a dose of 2000mg/kg. The oral LD_{50} of these formulations were therefore, considered greater than 2000mg/kg, b.wt.

Hepatoprotective activity evaluation

Effect on body weight

No significant body weight changes were observed among all the groups during the study period(Table-3).

Effect on serum ASAT, ALAT, ALP and TP

The results are given in Table-4. All the treatment groups show, a significant dose dependent protection against paracetamol induced increase in the serum ASAT, ALAT and ALP (P<0.05) except Group5 animals treated with low dose of GTE. In the case of TP, all the treated groups significantly prevented the paracetamol induced decrease in the serum total protein levels (p<0.05).

Effect on liver GSH, SOD, Catalase, GPx and lipidperoxide levels

The results are given in Table5. All the treatment groups show, a significant dose dependent protection against paracetamol induced decrease in liver GSH and SOD (p<0.05) except Group5 animals treated with low dose of GTE which shows a non-significant protection. In the case of catalase, only the Group 7&8 animals treated with low and high dose of ATG show significant protection (p<0.05), whereas the other treatment groups showed only a non-significant protection (p>0.05). In the case of GPx, all the treatment groups showed a significant dose dependent protection (p<0.05) except Group 3&4 animals treated with low and high dose of AT, respectively.

Effect on liver histopathology

The histopathological analysis of liver tissue of animals treated with AT, GTE and ATG reveal a dose dependent protection against paracetamol induced changes such as, fatty degeneration, lymphatic infiltration and necrosis. The higher protection was seen with ATG treated groups which show a near normal appearance of liver tissue (Fig. 1).

Discussion

Paracetamol produces liver injury through complex sequences of events which are depicted in Fig. 2. The oxidative stress resulting from the paracetamol toxic metabolite N-acetyl-para-benzoquinonimine (NAPQI) and subsequent glutathione (GSH) depletion are the major events which result in tissue damage(8-16). The biochemical changes associated with paracetamol toxicity include altered blood urea nitrogen, total protein, AST, ALT, ALP, glucose, bilirubin and creatinine levels and altered oxidative stress markers (lipid peroxide levels, GSH, SOD, Catalase, GP_X, etc.) (9, 17-19).The liver histopathological changes associated with paracetamol toxicity include centrilobular congestion, and hepatocellular degeneration and necrosis(9, 19). In the present study, similar biochemical and histopathological changes were observed with control rats treated with paracetamol alone (Table-4& 5 and Fig.1). However, pre-treatment with AT, GTE and ATG show a significant dose dependent protection against paracetamol induced changes (Table 4 & 5 and Fig.1). AT is a multi-vitamin and multi-mineral formulation and these components reported to possess antioxidant and hepatoprotective properties, either alone or in combination. Vitamin A, C, D and E and minerals such as Cu, Fe, Mn, Se, I, Mo, Co and Zn have shown beneficial effects against various toxicant

(including paracetamol) induced liver damage (20-31). Various studies report the hepatoprotective benefits of GTE and some reports correlate it to its major components, caffeine and catechins (32-44). In this study, we also observe that the combination formulation, ATG, shows a superior hepatoprotection compared to AT and GTE alone even at lower equivalent doses indicating a possible synergistic effect between the ingredients of AT and GTE. Previous studies have reported the synergistic association between GTE, vitamins, minerals and other antioxidant compounds (45-51). The superior hepatoprotective activity of ATG, therefore, may be attributed to these properties.

Conclusion

In conclusion, the present study clearly demonstrates hepatoprotective activity for AT, GTE and ATG against paracetamol induced liver injury in rats. When compared to individual formulations (AT & GTE) the combination formulation, ATG, shows superior hepatoprotection even at lower equivalent doses indicating synergistic association between the components of AT and GTE.

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Table 1: Composition of AT and ATG formulation

	AT (%w/w)	ATG (%w/w)
Vitamin C	4.76	4.76
Vitamin B3	1.90	1.90
Vitamin E	1.19	1.19
Vitamin B5	0.36	0.36
Vitamin B2	0.19	0.19
Vitamin B1	0.17	0.17
Vitamin B6	0.12	0.12
Vitamin B9	0.02	0.02
Vitamin H	0.018	0.018
Folic Acid	0.018	0.018
Vitamin A	0.476	0.476
Vitamin B12	0.0001	0.0001
Vitamin D3	0.0006	0.0006
Zinc	1.19	1.19
Magnesium	0.36	0.36
Manganese	0.03	0.03
Iodine	0.01	0.01
Copper	0.003	0.003
Selenium	0.003	0.003
Chromium	0.003	0.003
Green tea extract	-	5.95
Excipients	89.18	83.23

AT: Actilife tablet**; ATG:** Actilife tablet with green tea extract

Table 2: Dose selection

Product Name	Human dose (60kg man)	Equivalent rat dose	Selected doses
AT	1 Tablet (980mg)/day	52mg/kg	Low dose:50 mg/kg and High dose 100mg/kg.
GTE	50mg/day	5.2mg/kg	Low dose:5.0 mg/kg and High dose10.0 mg/kg.
ATG	1 Tablet(980mg)/day	52mg/kg	#Low dose:40 mg/kg and #High dose 80mg/kg.

#: In the caseof ATG lower equivalent doses of40 mg (instead of50mg) and 80mg (instead of 100mg) are selected to find out whether the combination acts synergestically.

Table 3: Effect of AT, GTE and ATG on body weight

Group	Treatment	Body weight(g)		
		Day-0	Day-7	Day-14
1	Normal (0.5% CMC 10 ml/kg, b.wt.)	187.0±10.1	193.1±8.9	200.5±7.8
2	Control (0.5% CMC 10 ml/kg, b.wt.)	187.2±13.2	194.3±12.3	199.3±12
3	AT (50 mg/kg, p.o.)	189.7±11.5	196.2±10.2	200.3±9.9
4	AT (100mg/kg, p.o.)	187.8±13.3	195.2±12.7	201±13.9
5	GTE (5mg/kg, p.o.)	187.8±9.9	194.8±10.3	200±9.8
6	GTE (10 mg/kg, p.o.)	185.2±10.6	191.5±10.6	196.8±9.7
7	ATG (40mg/kg, p.o.)	188.3±14.4	194.3±13.5	199.2±14.6
8	ATG (80mg/kg, p.o.)	188.2±11.8	194.3±13.7	200.3±13.8

#p<0.05 when compared to G1, Normal and *p<0.05 when compared to G2, Control.

Table4: Effect of AT, GTE and ATG on serum ASAT, ALAT, ALP and TP.

Group	Treatment	ASAT(U/L)	ALAT(U/L)	ALP(U/L)	TP(g/dl)
1	Normal (0.5% CMC 10 ml/kg, b.wt.)	73.3±10.7	35.7±6.1	102.8±12.8	11.2±2.2
2	Control (0.5% CMC 10 ml/kg, b.wt.)	110.8±7.4#	62.7±6.6#	169.7±21.8#	6.7±1.4#
3	AT (50 mg/kg, p.o.)	92.0±16*	42.8±8.5*	131.8±14.9*	10±1.1*
4	AT (100mg/kg, p.o.)	86.5±7.8*	47.3±8.7*	136.7±17*	9.7±1.5*
5	GTE (5mg/kg, p.o.)	92.3±7.2*	59.7±8.6	148.7±20	8.2±1.6
6	GTE (10 mg/kg, p.o.)	92.2±8.1*	44.3±10*	138.3±7.4*	9.3±1.6*
7	ATG (40mg/kg ,p.o.)	87.7±8.8*	47.8±10.9*	134.0±16.6*	10.2±1.6*
8	ATG (80mg/kg, p.o.)	77.2±8*	43.2±7.1*	136.2±19.5*	10.3±1.2*

#p<0.05 when compared to G1, Normal and *p<0.05 when compared to G2, Control.

Table 5: Effect of AT, GTE and ATG on liver GSH, SOD, Catalase, GPx and MDA levels

Group	Treatment	MDA (nmol/mg protein)	GSH (nM/mg protein)	SOD (u/mg protein)	Catalase (nmol/min/mg protein)	GPx (U/mg protein)
1	Normal (0.5% CMC 10 ml/kg, b.wt.)	17.2±1.1	7.9±1.3	26.6±2.5	105.4±13.8	14.5±2.5
2	Control (0.5% CMC 10 ml/kg, b.wt.)	39.6±1#	4.8±0.8#	14.1±1.3#	61.2±13.1#	9.9±3.0#
3	AT (50 mg/kg, p.o.)	34.0±2.3*	6.5±0.5*	20.2±1.5*	65.3±9.1	11.3±1.5
4	AT (100mg/kg, p.o.)	34.7±5.3*	7.1±0.3*	22.2±2.2*	67.7±13.3	11.6±1.8
5	GTE (5mg/kg, p.o.)	36.0±3.2	5.1±0.8	19.0±1.7*	65.0±11.9	14.9±2.2*
6	GTE (10 mg/kg, p.o.)	34.3±1.2*	6.8±0.8*	19.7±2.2*	61.6±9.3	13.3±2.3*
7	ATG (40mg/kg, p.o.)	30.3±2.2*	7.3±0.8*	23.4±2.2*	84.1±9.6*	15.1±1.5*
8	ATG (80mg/kg, p.o.)	29.9±2.1*	7.7±0.4*	22.8±1.9*	82.3±9.8*	14.6±1.9*

#p<0.05whencomparedtoG1, Normal and *p<0.05whencomparedtoG2, Control

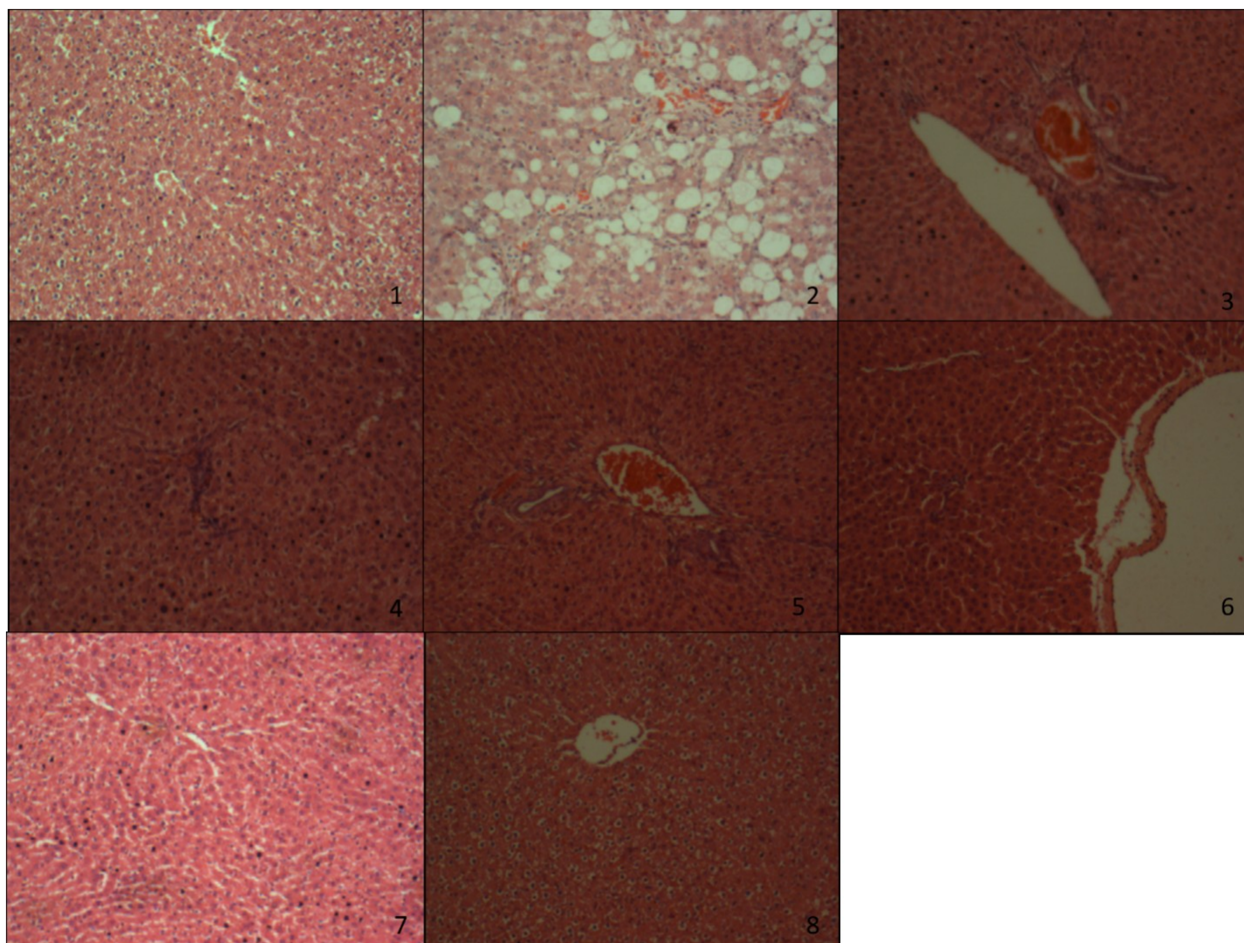

Fig. 1: Effect of AT, GTE and ATG on liver histopathology (H&E 10x)

1: Group 1(Normal) showing the normal appearance of the hepatic parenchyma including the portal areas. **2:** Group 2 (Control) treated with paracetamol alone showing severe degeneration and necrosis of hepatocytes. Severe neutrophil infiltration fatty degeneration and inflammation around centrilobular and portal triad regions. **3&4:** Group 3 and 4 treated with low and high dose of AT showing a significant hepatoprotection with minimal degeneration and necrosis of hepatocytes and minimal degree of neutrophil infiltration and inflammation. **5&6:** Group 5 and 6 treated with low and high dose of GTE showing mild to moderate degree of hepatoprotection with moderate degree of degeneration and necrosis of hepatocytes and minimal degree of neutrophil infiltration and inflammation. **7&8:** Group 7 and 8 treated with low and high dose of ATG showing a significant hepatoprotection with near normal appearance of the hepatic parenchyma.

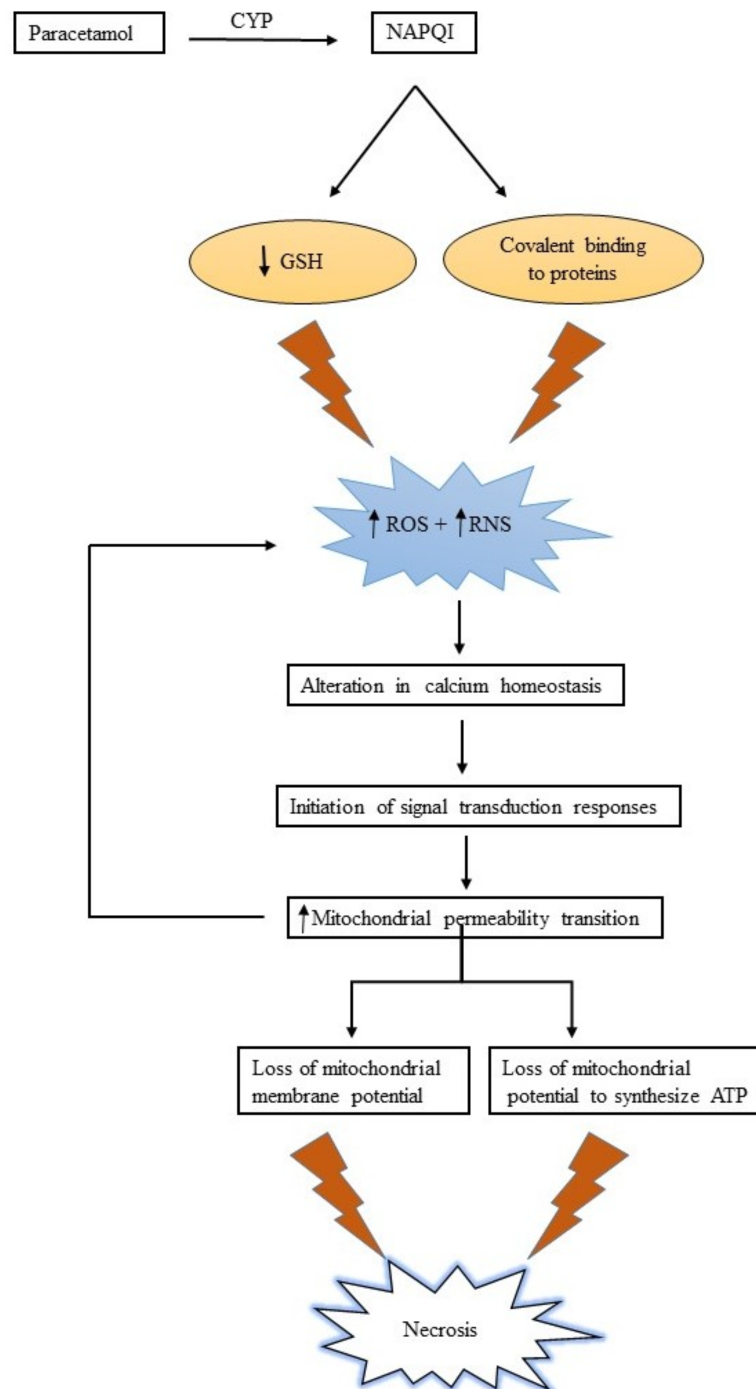

Fig.2: Mechanism of paracetamol induced hepatotoxicity

NAPQI: N-Acetyl-*p*-benzoquinone imine, **CYP**: Cytochrome P-450, **GSH:** Reduced glutathione, **ROS**: Reactive oxygen species, **RNS**: Reactive nitrogen species.