Anti-hepatotoxic Activities of Combination of Wedelolactone and Berberine against Paracetamol / Atractyloside induced Hepatotoxicity

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Abstract- **Pharmacological studies were performed for anti-hepatotoxic activity of BB, WDL and combination of BB with WDl against PCM and ATR induced hepatotoxicity in albino rats. LFTs were assessed with biochemical kits of ALT, AST, AB, T-Prot, AKLP and T-Bil. PCM / ATR induced hepatoxicity increased hepatic biochemical parameters due to hepatic necrosis. Elevated LFTs levels were correlated with necrosis, infiltration, broad infiltration of lymphocytes and kupffer cells, degeneration of hepatocytes (histopathology of PCM and ATR induced dose dependent hepatic lesions or necrosis). Treatment with berberine (100 mg/kg), wedelolactone (100 mg/kg), berberine in combination with wedelolactone (50 mg/kg each) and standard silymarin (140mg/kg) controlled hepato-toxicities (recovered animals to normalcy) with significant alleviation of LFTs. Hepatoprotective properties of BB and WDL (either alone or in combination) significantly reduced lipid peroxidation. Combination showed a remarkable anti-hepatotoxic activity but comparatively lesser anti-hepatotoxiceffects then Silymarin. Data was analyzed by ANOVA. Finally, it was found that BB in combination with WDL produced higher anti-hepatotoxic property then individual drug (BB / WDL) but lesser than Silymarin (Standard). Effects were induced by quenching of free radicals, attenuation depleted glutathione and membrane stabilization, inhibition of Phopholipase A2 and their use in combination is recommended for the treatment of DIH / DILI.**

Keywords: **Antihepatotoxic, atractyloside, berberine, glutathione, micrtomy, peroxidation, necrosis, paracetamol, wedelolactone.**

Introduction: Hepatotoxicity

Parabia *et al*., 2007, hepatotoxicity is a terminology used for liver damage and drugs / chemicals which cause liver - injury are known as hepatotoxins. Drug induced liver injury (DILI) / Drug-induced hepatotoxicity (DIH) are responsible for acute liver failures / hepatic coma or even death (drugs, toxins, and toxic herbs cause liver injury. (Haidry *et al*., 2014).

Liver Degenerative Disease (LDD)

Liver is vital organ of vertebrates and master chemist which regulates glycogenolysis; gluconeogenesis; proteolysis; lipogenesis and and excrete bile which excretes waste products (detoxification of drugs / xenobiotics). Homeostatis affected if liver functions got impaired. Dienstag *et al*., 2001 reported LDD accounts for 3.5%-9.5% of all ADRs and up to 14.7% of fatal adverse reaction (highly vulnerable; metabolism of exogenous compounds, drugs and toxic metabolites) (Sudipta *et al*., 2012). Stickel & Schuppan (2007), summarised that allopathic treatments lead to ADRs which cause hepatic damage.

Drug Induced Hepatotoxicity (DIH)

Mohit *et al*., 2011, DIH / DILI is a leading cause for termination of drug development in preclinical and clinical phases and affects a large population throughout the globe as liver is the master chemist of the body (highly vulnerable to damage by drugs and toxic metabolites). The Risk Factors for DILI/ DIH include race (blacks more susceptible to isoniazid; P-450 enzymes variation), age (old age population is at high risk of liver disaes due to poor blood-flow, poor drug - protein binding, interactions, poor clearance and decreased), sex (females are more prone than males for unknown reasons), genetic factors ((idiosyncratic reactions; gene encodes P-450 protein), alcohol consumption (alcoholics are more prone to liver injury due to depletion of glutathione), drug formulation (anti-HIV and anti-viral drugs liver disease; long acting drug / prolonged therapies cause hepatic injury), other co-morbidities (patients become more susceptible to drug reactions due to low glutathione depletion) (Kashaw *et al*., 2011).

Hepatotoxicants: Thonda *et al*., 2012 summarised that hepatotoxins are the substances whichcause liver damages (Table 1)

Table 1: List of Hepatotoxicants

Hepatotoxicants category	Examples	Hepatotoxicants category	Examples
Natural Products		Drugs	

Amanita phalloides	• Amatoxins like α-amanitin phallotoxins	Anaesthesia	• Halothane
Aspergillusflavus	• Aflatoxins	Antibiotics	• Amoxicillin • Clavulanate
Cyanobateria	• Microcystins • Nodularins	Anti-convulsants	• Valproate • Phenytoin
Ecreinascidia turbinate	• Ectenascidins	Anti-coagulants	• Heparin • Warfarin
Eupatorium adenophorum	• 9-oxo-10,11-dehydroagera phoroe, a sesquiterpinet annin	Antiretroviral	• Protease inhibitors (PIs) • NRTIs • NNRTIs
Fusarium moniliforme	• Fumonisin	Aniline analgesics	• Acetaminophen
Industrial toxins	• Heavy metals (arsenic, lead,mercury) • Vinyl chloride • Trichloroethylene	Anti- hyperlipidaemias	• Atorvastatin • Fenofibrate
Lantana camara	• Lantadenes	Anti-tuberculosis	• Isoniazid • Rifampicin
Penicilliumrubrum	• Rubratoxins	Anti-malaria	• Iminoquinone(amoduaquine)
Phompsis leptostromiformis • Phomopsin		Chemotherapy	• Imatinib • Nilotinib
		Corticosteroids	• Glucorticoids • Anabolic androgenic steroids
		NSAIDs	• Aspirin • Diclofenac
		Alcohol	• Alcohol

Toxicity of Herbal Medicines

Vandana (2005), summarized that herbal medicines have been portrayed as "wonder drugs" (first category) and second category as "medicinal herbs" - dosage and rationale for use should emphasized as they are stronger and used for specific conditions. Bos *et al.*, 1997 found that "poison herbs" (third category) produced acute or chronic toxicity (Figure 1-2).

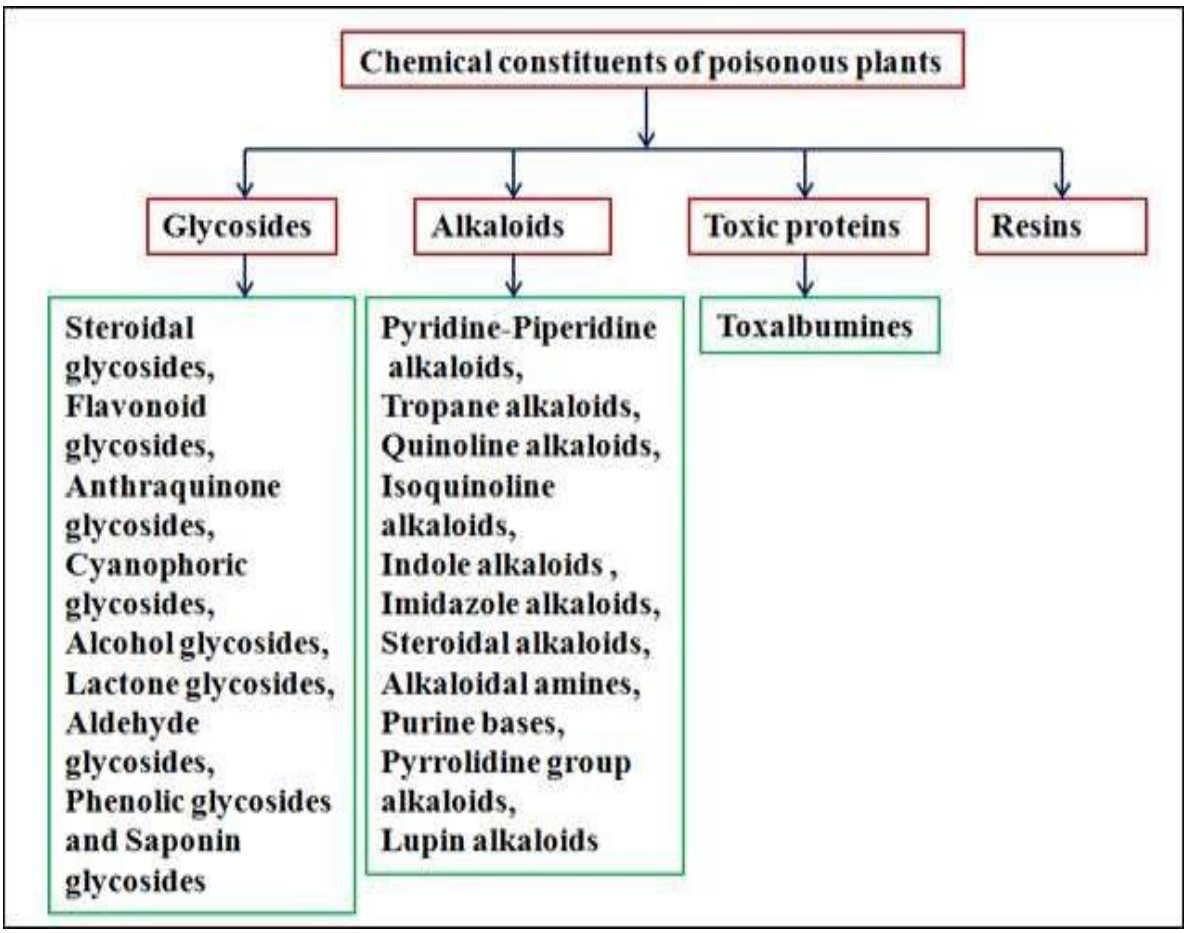

Figure 1: Classification of poisonous plants.

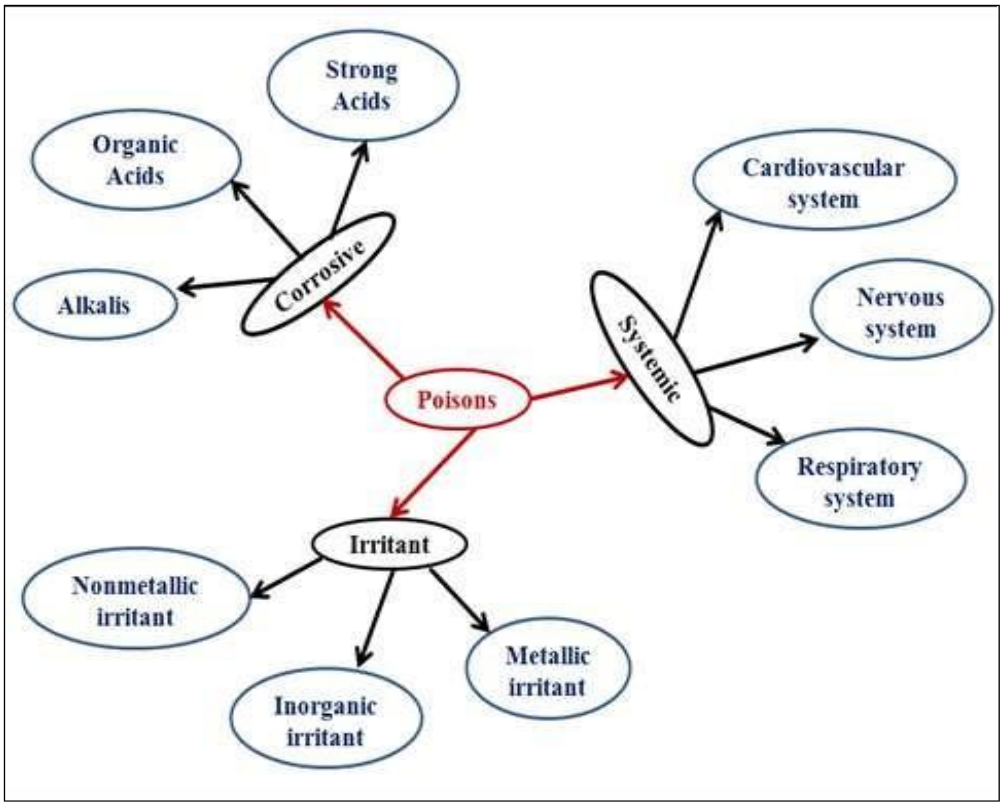

Figure 2: Types of poisons according to nature of poisoning.

Chukwuebuka *et al*., 2019, plants which produce toxic phytochemicals and lead to death e.g. *Atropa belladonna*, *Ricinus communis*, *Dieffenbachia Schott*, *Cicuta maculata*, *Hippomane mancinella*, *Abrus precatorius*. Some plants historically used by herbalists are generally understood as potentially poisonous. Poisonous plants are as follows:

- *Water Hemlock (Cicuta maculate*)
- *Castor Bean* (*Ricinus communis*)
- *Deadly Nightshade* (*Atropa belladonna*)
- *Rosary Pea (Abrus precatorius; Crab's Eye)*
- *Oleander* (*Nerium oleander*)
- English Yew (*Taxus Baccata*)
- Dieffenbachia

Herbal Drug Interactions

Adriane, 2000 illustrated that herbal interactions with drug increase or decrease pharmacological effects (Table 2-3). Concurrent use of herbs may mimic, magnify, or oppose the effect of drugs. (De Smet, 1996).

Table 2: Drug interactions with herbal medicines.

Herbal product	Interacting drugs
Ginseng	Warfarin
Kava	Sedatives, sleeping pills, antipsychotics, alcohol
Ginkgo biloba	Ticlopidine (Ticlid), Aspirin, warfarin (Coumadin), clopidogrel (Plavix), dipyridamole (Persantine)
St. John' s wort	Antidepressants
Ephedra	Caffeine, decongestants, stimulants

Table 3: Allopathic and Herbal Drug Interactions / ADRs.

S. No.	Medicine	SPMs	ADR
1	Essentiale-L	Essential phospholipids, linoleic acid, unseat fatty acid, Lecithin.	Nausea, stomach upset skin rash, contraindicated in pregnancy
2	Mecolin	Tricholine Citrate, Sorbitol	Abdominal pain, Diarrhea, Flatulence, Hyperuricaemia, Urticaria
3	Sorbiline	Sorbitol , Tricholine citrate	Abdominal pain, Diarrhea, Urticaria Hyperuricaemia, Lactic acidosis,
4	Hepa Merz	L-Ornithine, L-Aspartate	Hyperuricaemia, Lacrimation, Skin rashes, Sneezing
5	Livosil Forte	Silymarin, Thiamine, Riboflavin, Pyridoxine, Pantothenic acid	Bloating, dyspepsia, irregular stools, laxation.

Toxic Effects of herbal medicines on Body Organs

Eisenberg *et al.*, 1998 reported toxic effects of herbal medicines (Table 4):

Table 4 : List of herbal drugs having toxic effects.

Drugs with Toxic Effect	Toxic Constituent
Piper mysthstiycum	Pipermthystine
Gingko biloba	Ginkgolic Acids
Comfrey	Pyrrolizidine Alkaloids
Sassafras and Mutmeg	Safrole
Mugwort and Wormwood	Thujone
Ephedra	Ephedrine
Teucrium chamaedrys	Diterpenes Teucrin A
Matricaria chamomile	Coumarin
Hypericum perforatum	Hypericin
Valerian	Valepotraits
Morinda citrifolia	Anthraquinones
Callilepsis laureola	Atractyloside
Azadirachta indica	Azadirachtin
Senecio vulgaris	Pyrrolizidine Alkaloids
Solanum nigrum	Solanine
Calotropis procera	Whole Latex
Semecarpus anacardium	Phenols
Nicotiana tobaccum	Nocotine
Strychnos nuxvomica	Strychnine
Argemone maxicana	Argemone, Sangnuinarine
Plumbago zeylanica	Plumbagin
Abrus precatorius	Abrin
Crotalaria karagwensis	Alkaloids
Medicago sativa	Seeds (Autotoxicity)
Gallium odoratum	Coumarin
Parthenium hysterophorus L	Parthenin

Pathophysiology of Drug Induced Hepatotoxicity

Pathophysiological mechanisms of hepatotoxicity include both hepatocellularand extracellular mechanisms as follows:

- Drug toxicity mechanisms
- Apoptosis of hepatocytes
- Cytolytic T-cell activation
- Disruption of the transport proteins

- Mitochondrial disruption
- Bile duct injury
- Disruption of the hepatocyte
- Intrinsic or predictable drug reactions

Mohit *et al*., 2011 stated that hepatotoxins (e.g. carbon- tetrachloride, alcohol, pyrrolizidine alkaloids, mycotoxins and bacterial toxins) or drugs (e.g. paracetamol, anti-tuberculosis drugs e.g. rifampicin) in overdoses / prolonged therapy induce hepatotoxicity.

Herbals in the Treatment of Liver Diseases / Anti-hepatotoxic / Hepatoprotective Plants

Radha *et al*., 2005, treatment of hepatoxicity is an important issue of today's research domain (because of many allopathic drugs and their toxic metabolites lead tohepatic damage; Thyagarajan *et al*., 2002)

Some of the other plants reported as hepatoprotective in animals by Indian investigation during the last decades are *Picrorrhiza kurrora, Eclipta alba, Silybum marianum, Andrographis paniculata , Boerhavia diffusa, Cichorium intybus, Phyllanthus debilis, Tephrosia purpurea, Phyllanthus niruri, Phyllanthus embelica, Ocimum sanctum, Acacia catechu, Terminalia arjuna, Curcuma longa, Taraxacum officinale, Azadirachta indica, Capparis spinosa, Daucus carota, Solanum nigrum, Geophila reniformis, Phyllanthus amarus, Moringa olifera, Glychyrrhiza glabra, Swertia chirata, Chelidonium majus, Chionanthus virginicus , Sida cordifolia, Fumaria indica, Terminalia nigrum* and *Withania somnifer*a. These plants have been formulated together in different doses and combinations to achieve maximum synergistic antihepatotoxic / hepatoprotective activity.

Berberine

Xia *et al*., 2010, berberine (Figure 3) is a bitter, yellow colored (shows a strong yellow fluorescence under UV light) quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids found in *Berberis Vulgaris* (Barberry), *Berberis aristata* DC (goldenseal), oregon grape, and goldthread. Berberine is used as dye, antibiotic against bacteria, viruses, fungi, protozoans,helminthes, and chlamydia, and in histology for staining heparin in mast cells.

Berberine Hydrochloride ; *Synonym*: Berberine Chloride

Molecular formula $C_{20}H_{18}NO_4+$ Molar mass 336.36122 g/mol

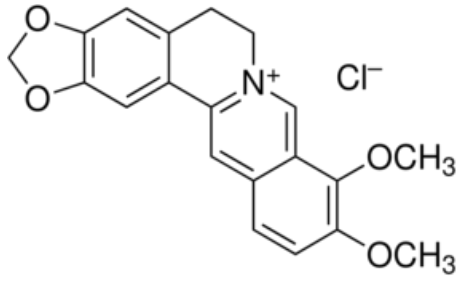

Figure 3 : Chemical Structure of Berberine.

Wedelolactone

Wedelolactone (furanocoumarin; $C_{16}H_{10}O_7$; mol. wt. of 314.3; yellow-green solid; Figure 4) compound as a coumestan that occurs in *Eclipta alba* (false daisy) and in *Wedelia calendulaceae***.** Wedelolactone (7-methoxy-5,11,12-trihydroxy-coumestan) possess hepatoprotective, sedative, muscle-relaxant, anxiolytic, anti-stress activities. (Govindachari *et al*., 1956; Neerja *et al*., 2008; Wagner *et al*. 1986).

Molecular formula $C_{16}H_{10}O_7$Molar mass 314.2464 g/mol

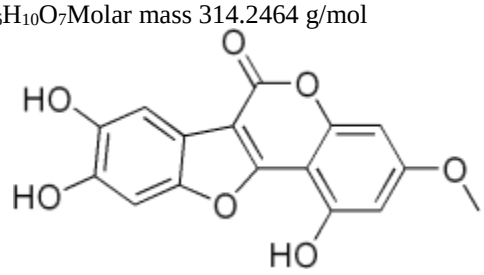

Figure 4 : Chemical Structure of Wedelolactone.

Paracetamol (PCM)

Mitchell *et al*., 1973 summarised that Paracetamol (PCM; Synonym:acetaminophen; N-acetyl-p-aminophenol; para-

aminophenol derivative phenacetin and acetanilide). PCM is "over-the-counter" (OTC) which occupied 50 -60% of "OTC" analgesic market. Shah *et al*., 2011 found that paracetamol excessive use or overdose produces acute liver damage (fatal necrosis, amino-transferases and billirubin elevation with centilobular necrosis, glutathione depletion (Wright *et al*., 1973; Mukherjee, 2002)

Prescott, 1986 reported the self poisoning with PCM (estimated 41,200 cases poisoning in 1989 to 1990, mortality (0.40%), alone or in combination with other drugs, is becoming increasingly common. Paracetamol reduces the oxidized form of the COX enzyme and after toxic doses (plasma paracetamol concentrations of up to 280 mg/l), protein binding varied from 8 to 43 % (10 and 20 % PCM bound to RBC). Sulfate conjugation (sulfation) may account for 20–40%; GSH conjugation (less than 15%).

Atractyloside (ATR)

Lefranc (1868) isolated Atractyloside from the roots of *A. Gummifera* and *Callilepsis laureola* by Popat *et al.*, 2001, *Widelia glauca* by Schteingart *et al.*,1984and coffee plants (Figure 5).

Molecular Formula: $C_{30}H_{44}Na_2O_{16}S_2$ Formula Weight: 770.77

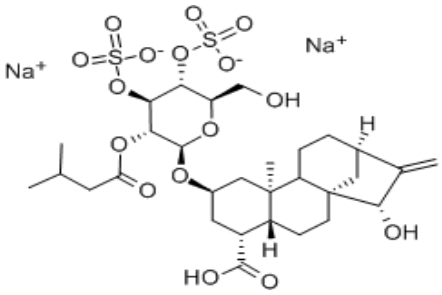

Figure 5: Atractyloside Sodium Salt.

Synonyms: Glycoside; Atraotydin; Atractyloside; Atractyloside sodium salt; *Atractyloside* sodium salt from *Atractylis gummifera.*

Steenkamp *et al.*, 2004, Atractyloside is also found to be present in *Atractylodes lancea* has a strychnine-like action. Belitz *et al.*, 2009 the results show that the ATR content in the herb is 8.98 thousand ppm, the ATRactylodes ATR content to 9230 ppm. Karl *et al.*, 2006, ATRthe toxic principle is commonly found in *Callilepis laureola.* Candy *et al.*, 1977, ATR is also found in *Coffea arabica* Linn. (Family Rubiaceae) / coffee beans. The South African traditional remedy *Impila* (*Callilepis laureola*) contains the mitochondrial toxin ATR (Brookes *et al.*, 1983). Yahara *et al.*, 1989, Cocklebur (*X. strumarium*) is an herbaceous annual plant with worldwide distribution. The roots and seeds of the plant are reported to atractyloside, which is highly toxic to mammals.

Atractyloside (ATR) Pharmacology and Toxicology

Stewart, 1998 reported that ATR cause nervous breakdown and and even death (high moratality rate). Van *et al.*, 2002 showed that Atractyloside (ATR) is a mitochondrial toxin (inhibits oxidative phosphorylation) and extremely toxic.

Materials and Methods

Xia *et al*., 2010, Berberine (BB) an isoquinoline alkaloid, isolated from tree turmeric *Berberis aristata* DC (goldenseal; Family: Berberidaceae) possess significant medicinal uses and anti-hepatotoxic activities in experimental animals against various hepatotoxins. Chemically it is a bitter, yellow colored quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. Thorat *et al*., 2010, wedelolactone (WDL; furanocoumarin; $C_{16}H_{10}O_7$; mol. wt. of 314.3; yellow-green solid) is an organic chemical compound classified as a coumestan that occurs in *Eclipta alba* Linn. and in *Wedelia calendulaceae*. In the present investigation an attempt has been made to perform *in-vivo* pharmacological investigations of to investigate anti-hepatotoxic effects of berberine in combination with wedelolactone against paracetamol and atractyloside to establish the mechanism involved in anti-hepatotoxic activity. Biochemical parameters (like SGPT, SGOT, total protein, serum albumin, serum billirubin and alkaline phosphatase etc.), histopathological analysis of liver tissues were performed.

Anti-hepatotoxic Assessments

Animal study was approved with CPCSEA (IAEC) Form B. ProposalIEC/IAEC/2023/02 dated 17-02-2023

Animal Groups

Table 5: Animal Group with treatment schedule. (6 animals/group).

Activity Group	Treatment (dose and dosing schedule)

I : Normal	Water, ad libitum
II : Toxic Control	PCM : 1 gm/kg p.o.
III : Toxic Control	ATR : 100 mg/kg p.o.
IV: Drug Treated	PCM (1 gm/kg p.o.) for 21 days + WDL (100 mg/kg b.wt./day) for 07 days.
V: Drug Treated	PCM (1gm/kg) for 21 days + BB (100 mg/kg) for 07 days.
VI: Drug Treated	PCM (1gm/kg p.o.) for 21 days + WDL (50 mg/kg) with BB (50 mg/kg) for 07 days.
VII: Drug Treated	ATR (100mg/kg) for 21 days + WDL (100 mg/kg) for 07 days.
VIII: Drug Treated	ATR (100mg/kg) for 21 days + BB (100 mg/kg) for 07 days)
IX: Drug Treated	ATR (100mg/kg) for 21 days + WDL (50mg/kg) with BB (50 mg/kg) for 07 days.
X: Standard Drug / (Silymarin)	PCM (1gm/kg) for 21 days + Silymarin (140 mg/kg p.o for 7 days)
XI: Standard Drug / (Silymarin)	ATR (100 mg/kg) for 21 days + Silymarin (140 mg/kg) for 7 days

Group I : Animals were given water *ad libitum* and biochemical parameters (LFTs)were estimated. Liver issues were also taken for histopathological studies.

Group II : Animals of Group II were served as toxic control group (PCM Control Group1 gm/kg body weight p.o. daily for 21 days.

Group III : Animals of Group II were served as toxic control group (ATR Control Group 100 mg/kg body weight p.o. daily for 21 days.

Group IV to XI :Animals of Group IV to XI were given PCM / ATR to induce hepatotoxicity followed by by treatment with BB (100 mg/kg body) for 01 week (Group IV and Group VII), WDL (100 mg/kg) for 01 week (Group V and Group VIII), Berberine in combination with Wedelolactone (50 mg/kg of each; Group VI and IX), Silymarin (Standard) for 01 week (Group X and XI). (Table 6; Figure 6)

Table 6 : Anti-hepatotoxic activity of BB, WDL, BB in combination with WDL.

GROUP	SGPT	SGOT	SerumALB	T- Prot.	AKLP	T. Bil.
Group I (Normal)	32.2 □ 1.46	42.6 □ 1.68	3.42 □ 0.28	4.46 □ 0.34	17.12 □ 1.88	0.92 □ 0.16
Group II(PCM)	186.2 □ 3.82	158.4 □ 2.64	3.82 □ 0.42	4.68 □ 0.76	33.8 □ 2.4	1.42 □ 0.26
Group III(ATR)	188.4 ±6.18*	162.6 ±4.2*	3.95 ±0.48*	4.94 ±0.45*	34.2 ± 2.6*	1.86 ±0.20*
Group IV (PCM+ BB)	84.2 □ 2.62	86.2 □ 1.96	3.62 □ 0.24	3.62 □ 0.36	22.4 □ 1.24	1.12 □ 0.20
Group V (PCM+WDL)	90.7 □ 1.86	93.2 □ 1.42	3.72 □ 0.82	3.96 □ 0.44	24.2 □ 2.2	1.20 □ 0.22

Group VI (PCM+BB+ WDL)	79.4 □ 2.38	83.6 □ 1.42	3.48 □ 0.12	3.54 □ 0.32	21.2 □ 1.18	1.02 □ 0.16
Group VII (ATR+ BB)	87.4 □ 1.68	91.6 □ 1.24	3.56 □ 0.28	3.88 □ 0.32	22.6 □ 1.8	1.16 □ 0.14
Group VIII (ATR+WDL)	92.4 □ 1.48	95.4 □ 1.54	3.64 □ 0.84	3.98 □ 0.48	24.8 □ 2.4	1.18 □ 0.26
Group IX (ATR+BB+ WDL)	82.6 □ 2.62	84.8 □ 1.56	3.56 □ 0.28	3.68 □ 0.38	22.4 □ 1.26	1.10 □ 0.16
Group X (PCM+SMN)	68.2 □ 2.30	78.6 □ 1.72	3.42 □ 0.42	3.38 □ 0.34	19.2 □ 1.2	0.96 □ 0.14
Group XI (ATR+SMN)	78.2 □ 2.30	80.6 □ 1.71	3.48 □ 0.41	3.42 □ 0.37	19.8 □ 1.4	0.98 □ 0.18

Note: Values are Mean ± SE

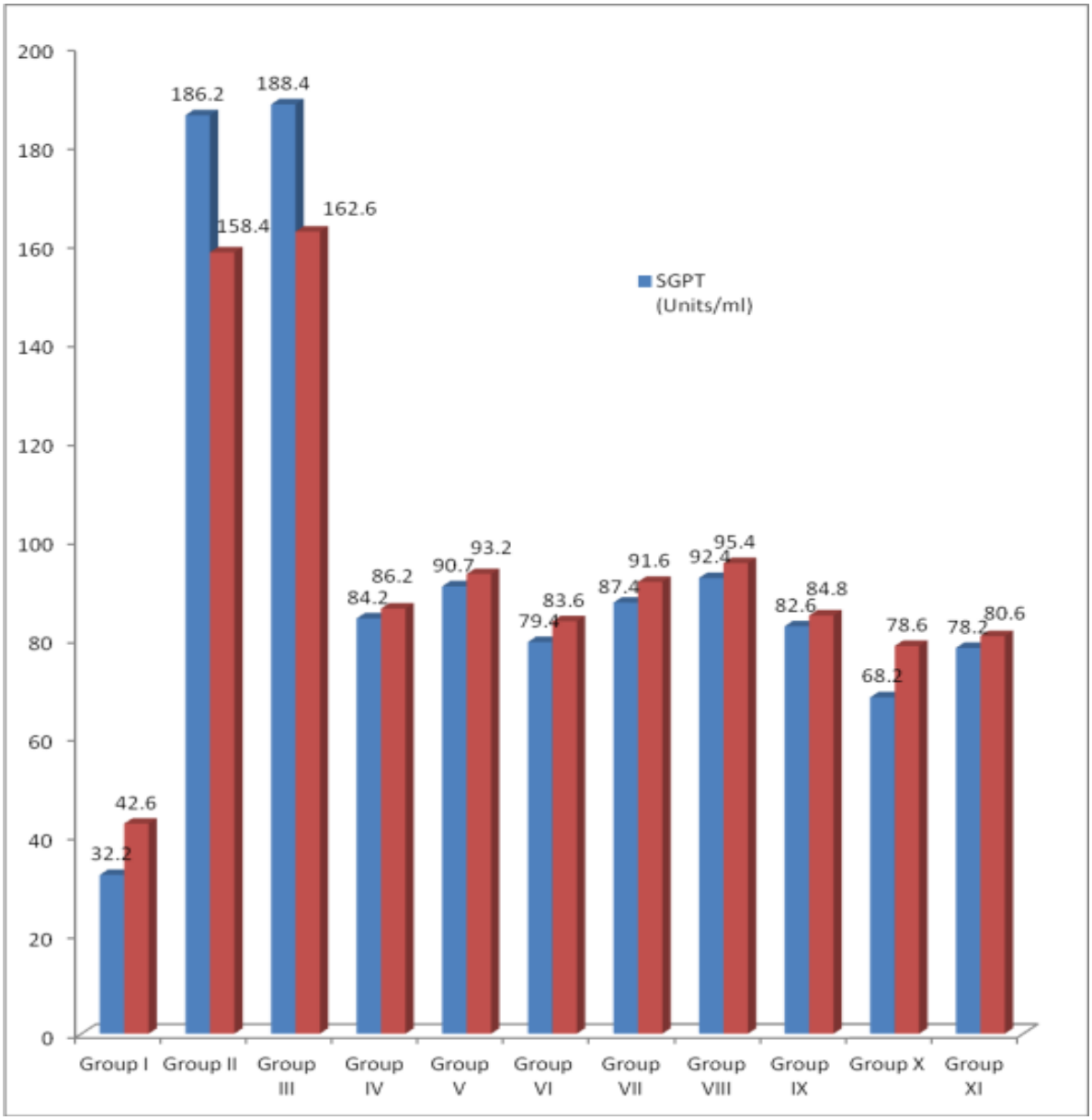

Figure 6: Effects of BB, WDL, BB in combination with WDL onSGPT and SGOT.

Results and Discussions

Pharmacological investigation was carried out for anti-hepatotoxic activity purified berberine, wedelolactone and combination of berberine with wedelolactone against PCM and ATR induced hepatotoxicity in albino wistar rats. SGPT (ALT), SGOT (AST), Abumin (AB), Total proteins (T-Prot), alkaline phosphatase (AKLP) and Billirubin (T-Bil.) were estimated. TS of normal liver tissues were prepared using the microtomy technique.

PCM / ATR induced hepatoxicity (increased hepatic biochemical parameters like ALT, AST, ALKP, T-Bil, AB, T-prot. etc.) due to necrosis of hepatic cells. Elevated level of LFTs parameters were correlated with hepatic lesions produced (necrosis, infiltration, broad infiltration of lymphocytes and kupffer cells, degeneration of hepatocytes). Histopathology showed that PCM and ATR induced hepatoxicity includes damaged anatomical architecture and caused severe dose dependent hepatic lesions or necrosis. One-week Treatment with berberine (Group V and Group VIII; 100 mg/kg), wedelolactone (Group IV and Group VII; 100 mg/kg), berberine in combination with wedelolactone (Group VI and Group IX; 50 mg/kg each) and silymarin (standard; 140mg/kg) recovered animals to normal anatomical architecture of hepatocytes (controlled hepato-toxicities) and produced significant alleviation of LFTs with reversal effects. Hepatoprotective properties of berberine and wedelolactone (either alone or in combination) significantly ameliorates lipid peroxidation. Combination showed a remarkable anti-hepatotoxic activity (Group VI and Group IX) but comparatively lesser anti-hepatotoxic effects then Silymarin (Group X-XI). Data of various enzyme activities was analyzed by ANOVA. Combination of BB and WDL was found produced higher anti-hepatotoxic property then the individual drug (BB/WDL) but lesser than the standard drug Silymarin (Table 6; Figure 6). BB in combination with WDL produced very good anti-hepatotoxic effects by inhibition of Phopholipase A2, quenching of free radicals, attenuation depleted glutathione and membrane stabilization. So, their use in combination is recommended in drug induced hepatotoxity.

Conclusions

In the present studies, berberine (BB) was separated from *Beberis aristata* (stem bark) and wedelolactone (WDL) from *Eclipta alba* (leaves). During hepatotoxic assessment, it was observed that BB in combination with WDL produced anti-hepatotoxic effects by inhibition of Phopholipase A2, quenching of free radicals, restoration / attenuation depleted glutathione and membrane stabilization. So the combination of both the drugs (BB and WDL) possessed higher anti- hepatotoxic property then the individual drug (BB / WDL) but lesser than the standard drug - Silymarin. So, their use is recommended against other hepatotoxins and much more elaborative studies are also proposed.

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