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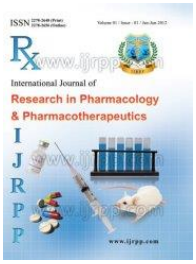

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TNF- α, (NF-κB) p65 expression & MPO restraint impact of Bacoside A, Withanolide A & Shatavarin IV in ulcer protection and ulcer healing

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ABSTRACT

In clinical practice peptic ulcer is the most prevalent disorder of gastrointestinal tract. A variety of antiulcer medications available, however all have the occurrence of relapses, adverse effects and reactions to medicines. The quest for new molecules was expanded to herbal drugs which give better protection and decreased relapse. The current research is to determine ability of Bacoside A, Withanolide A and Shatavarin IV ulcer-protective and ulcer-healing treatments. The impact of distinct phytoconstituents on expression of nuclear factor kappa B (NF-ÿB) p65, levels of gastric myeloperoxidase (MPO), inflammatory cytokines: factor-α (TNF-α) level of tumor necrosis, level of anti-inflammatory interleukin-10(IL-10), level of gastric mucosal apoptosis caspase-3 and parameters of gastric oxidative stress were established against gastric injury induced by ethanol in rats. Bacoside A and Shatavarin IV pretreatment (200µg/ml/kg), withanolide A (20µg/m/kg) mitigated the intensity of gastric inflammation by curtailing the levels of myeloperoxidase (MPO) and tumor necrosis factor-α (TNF-α) along with the expression of nuclear factor kappa B (NF-µB) p65. This also raised the levels of anti-inflammatory interleukin-10(IL-10). With regard to gastric mucosal apoptosis, phytoconstituetns inhibited caspase-3 activity meanwhile; it halted gastric oxidative stress via lipid peroxide inhibition with concomitant glutathione (GSH), glutathione peroxidase (GPx) and total antioxidant ability (TAC) enhancement. These results emphasize the gastro protective and gastric healing activities of phytoconstituents were mediated by concerted multi-pronged activities, including suppression of gastric inflammation, apoptosis, and oxidative stress, in addition to boosting the antioxidant parameters.

Keywords: Bacoside A, Withanolide A, Shatavarin IV, Tumor Necrosis Factor-α (TNF-α), Interleukin - 10, Caaspase-3.

INTRODUCTION

Ulcer is a major gastrointestinal system disease with various aetiologies affecting 10 per cent of the world population. Repeated intake of alcohol, smoking, prolonged stress, chronic use of non-steroidal anti-inflammatory medications and bacterial Helicobacter pylori infection are primary causes of inflammatory peptic ulcer, mucosal bleeding and abdominal pain in patients [1, 2]. Such ulcers can develop when the gastro protective (mucus, bicarbonate and prostaglandins) and aggressive (acid, pepsin, bile salts, and H.pylori bacteria) imbalances occur [3].

The recent approach to peptic ulcer is controlled for successful heal by inhibiting gastric acid secretion, fostering gastro-protection, blocking apoptosis and stimulating epithelial cell proliferation. The conventional drugs used in the treatment of ulcer include histamine receptor antagonists, prostaglandin analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics, but most of these drugs produce undesirable side effects or drug interactions, and may even alter the body's biochemical mechanisms upon chronic use. Therefore, herbal medicinal products are typically used in such chronic cases where drugs are needed for long periods of use [4].

Bacoside A is a chemical compound mixture, known as Bacosides Isolated from Bacopamonnieri [5]. The principal constituents include saponinsbacoside A3, bacopaside II, bacopasaponin C jujubogenin isomer [6, 7]. For its possible neuroprotectivity [8-15] the mixture was tested in in vitro experiments and animal models. Bacoside A3 inhibits P-glycoprotein (P-gp), an ATP-binding cassette transporter, decreasing efflux4-fold over in vitro [16]. An extract containing bacoside A3 and bacopaside II, administered for eight days at a dosage of 10-30 mg / kg, decreases depression in mice caused by opioid withdrawal using the forced swim test [17]. Bacoside A3 has also antioxidant properties in rats following liver and kidney damage caused by street heroin [18].

Withanolidesis a gaggle of a minimum of 300 present steroids built on an ergostane skeleton [19]. Structurally, Withanolide A is an isolated, natural product from medicinal plant W. Somnifera with antioxidant and neuroprotective impact [20]. Withanolide A reverses hypoxia-mediated neurodegeneration by restoring hypoxia-induced glutathione depletion within the hippocampus of mice [21].

Shatavarin IV, the sarsasapogenin glycoside, is the main saponin present in the Asparagus racemosus roots. This is an essential constituent of carcinogenic and antioxidant nutraceuticals and functional foods [22, 23]. Shatavarin IV is an anticarcinogen, illustrated by in vitro and in vivo studies [24].

MATERIAL AND METHODS

Animals

The 200-300 g average weight wistar albino rats used from home laboratory. The animals were held in animal house, Department of Pharmacology, Excel College of Pharmacy, Komarapalayam, under uniform environmental conditions (22-28oC, 60-70 percent ratio, 12 hr dark / light cycle). Standard mouse chow (SaiDurga Feeds and Foods, Bangalore, India), and water ad lib were given to the animals.

Experimental design and treatment protocol

Rats are individually placed in metabolic cages with large mesh raised floors to prevent coprophagy that interferes with gastric ulcer induction. The animals fasted on free access to water for 24 hours. Instead, the water was withdrawn 1 h before ethanol administration. Thirty six rats allocated randomly into six groups (6 rats per group). Group I served as Control group, normal rats which received oral vehicle (0.5 percentage carboxymethyl cellulose; 5mL/kg), Group II served as Ethanol group administered with single intra gastric dose absolute ethanol (5ml/kg) + oral vehicle (0.5 percentage carboxymethyl cellulose) for 7 days and the last dose of the vehicle given60 minutes before ulcer induction, Group III served as Ethanol + Sucralfate group received a single intragastric dose of ethanol + an oral Sucralfate (SUCR) (100 mg/kg) 60 minutes before ulcer initiation, Group IV served as Ethanol + Bacoside A group, received a single intragastric dose of ethanol + oral Bacoside A (200µg/ml/kg) for 7 days and the last dose of Bacoside A administered 60 minutes prior to the induction of ulcer, Group IV was Ethanol + Withanolide A group, received a single intragastric dose of ethanol + oral Withanolide A (20µg/ml/kg) for 7 days and the last dose of Withanolide A administered before 60 minutes of ulcer induction, Group V was Ethanol +

ShatavarinIV group, received a single intragastric dose of ethanol + oral Shatavarin IV (200µg/ml/kg) for 7 days and the last dose of Shatavarin IV administered 60 minutes before ulcer induction.

Gastric ulcer induction

Gastric mucosal injury was induced by the use of a single intragastric dose of absolute ethanol (5ml / kg) administered by or gastric intubation [25-28]. Instead of ethanol the control group got the same amount of saline. 60 minutes later Animals were euthanized dissection of stomach made immediately.

Collection of tissue and preparation

The animals were euthanized 60 minutes later of ethanol instillation under intense ether anesthesia. Then laparotomy performed immediately, removal of stomach and opened along the longer curvature, in order to remove the gastric contents and blood clots it was rinsed with normal saline. Stomach blotted dry then dichotomized, first portion was immersed in 10 percent buffered formol saline for the evaluation immune histo chemical evaluation of NF-κB and histopathology and, while the other second portion was divided into 2 portions and stored at-80 ° C for the investigation of biochemical parameters. The another side, one portion was homogenized for the calculation of MPO, TNF-α,IL-10, caspase-3 in 10 volumes of lysis buffer (200mMNaCl, 5mM EDTA, 10 mMTris, 10% Glycerin, 1 mM PMSF, 1 mg / ml Leupeptin and 28 mg / ml aprotinin, pH 7.4). The second component homogenized for determination of oxidative stress markers and cellular defenses (MDA, NO, GSH, GPx and TAC) in 10 volumes of ice-cold phosphate buffer (100 mM, pH 7.4);

Detection of immunohistochemical NF-KB P65

For antigen recovery, 5 µm thick paraffin-embedded tissue samples were rehydrated in xylene and then in graded ethanol solutions, and heated for 5 min in citrate buffer (pH 6). Blocking performed in Tris buffered saline (TBS) with 5 percent bovine serum albumin (BSA) for 120 minutes. Then the samples were incubated overnight at 4 ° C with primary polyclonal rabbit anti-NF-ÿB p65 (Santa Cruz Biotechnology Inc, CA, USA) at a concentration of 1 µg / ml at 5% BSA in TBS. Washing of the slides made with TBS and incubated using Vector Elite ABC package (Vector Laboratories, Burlingame, CA, USA), using secondary goat anti-rabbit IgG. Eventually, the parts are washed with TBS and using 3.3'-diaminobenzidine (DAB Substrate Kit, Vector Laboratories Inc) tetra hydrochloride the immunoreaction was visualized. Hematoxylin was used to counter stain and a blinded observer observer the slides under a light microscope (Leica Microsystems, Germany).

Inflammatory cytokines (TNF-A AND IL-10) &gastric MPO activity

Neutrophil infiltration marker myeloperoxidase (MPO) Activity was calculated by the HK105 kit (Hycult Biotech, Uden, Netherlands) By using the manufacturer's R&D systems ELISA kits (MN, USA)s the concentrations of TNF-α and IL-10 were determined. MPO as well as cytokine assays use quantitative immunoassay antibody sandwich technique with pre-coated specific antibodies on the micro plate. The samples control and standards were pipetted into the wells and the immobilized antibody binds the rat cytokines. An enzyme-linked secondary antibody specific for rat MPO, TNF-α or IL-10 was applied to the wells after any unbound substances were washed away. The assay is stopped following color development, and the absorbance was read at 450 nm. In the initial step the color intensity was proportional to the sum of the corresponding target set. The outcome for MPO is reflected as (U / g tissue), whereas the cytokine content was presented as pg / g tissue.

Activity of caspase-3

Caspase-3 response calculated colorimetrically with the help of manufacturer's R&D systems package as directed. The 405 nm (BiochromAsysmicroplate reader, UK) Caspase-3 cleaves the labeled substrate DEVD-pNA (acetyl-Asp-Glu-Val-Asp p-nitroanilide) freeing chromophorepNA. Effects represented as a fold change of caspase-3 activity.

Concentration of lipid peroxides

Determination of lipid peroxides, expressed as malondialdehyde (MDA), is performed according to assay of the thiobarbituric acid29. The reaction continued for 30 min in 15% w / v trichloroacetic acid, 0.375% w / v thiobarbituric acid, and 0.25 N

HCl at 90°C. The precipitate was extracted by centrifugation after cooling; at 535 nm the absorbance was registered. The findings were described via nmol / g tissue.

Levels of GSH

As previously described, gastric GSH levels were determined30, gastric proteins were precipitated with 10 percent trichloroacetic acid, and the colored component was produced with a solution of 10 mM DTNB (5, 5'-dithiobis2-nitrobenzoic acid) in 0,3 M phosphate. At 412 nm the absorbance was estimated and the findings are expressed in the form of nmol / g tissue.

Activity of GPX

Activity of the gastric GPx is calculated using the manufacturer's instructed Sigma-Aldrich assay kit (Sigma-Aldrich, St. Louis, MD, USA). The package uses an indirect GSH oxidation-based determination by GPx, which was then coupled by recycling the oxidized GSSG by glutathione reductase and NADPH. At 340nm the decrease in the absorbance of NADPH recorded. The amount of enzyme oxidizing one micromole of NADPH at 25 ° C per min defined as one unit of enzyme. Gastric homogenates protein content was determined [25].

TAC determination

The TAC was calculated using complete antioxidant assay kit from Cayman, as indicated by the manufacturer. The ability of gastric antioxidants to prevent oxidation of metmyoglobin-induced ABTS (2, 2-azino-di-[3-ethylbenzthiazoline sulphonate]) was compared with Trolox, an equivalent water-soluble tocopherol. At 405nm the oxidized product quanity was measured the amount of oxidized products is measured at 405 nm and the TAC is expressed as Trolox equivalent µmol / g tissue.

RESULTS

Detection of immunohistochemical NF-κB p65

Expression of NF-κB p65 is a symptom of presence of chronic inflammation. The control group animals treated with 0.5% CMC at the dose of 5ml/kg dose not expressed NF-κB p65. The ethanol group animals treated with 5ml/kg of ethanol showed the copious expression of NF-κB p65, the animals treated with Ethanol + Sucrlafate at the dose of 5ml/kg +100mg/kg also does not expressed any NF-κB p65. Treatment with the phytoconstituents showed that reduced expression of NF-κB p65 which is comparable to the standard drug, there by the treatment with phytoconstituents decreases the chronic inflammation Table 1.

Table 1: Phytoconstituents impact on NF-KB p65 expression

Group	Dose	NF-KB P65 Expression
Control (0.5%CMC)	5ml/kg	-
Ethanol	5ml/kg	+++
Ethanol + Surlafate	5ml/kg +100mg/kg	-
Ethanol +Bacoside A	5ml/kg +200µg/ml/kg	+
Ethanol + Withanolide A	5ml/kg +20µg/ml/kg	+
Ethanol + Shatavarin IV	5ml/kg +200µg/ml/kg	+

INFLAMMATORY CYTOKINES (TNF-ALPHA AND IL-10) AND GASTRIC MPO ACTIVITY

Gastric MPO activity

Treatment with ethanol at the dose of 5ml/kg enormously increases the gastric MPO 8.14±1.44 U/g tissue; Pretreatment with the sucralfate at the dose of 100mg/kg significantly decreased the levels of gastric MPO i-e 4.25±0.13 U/g tissue. Pretreatment with phytoconstituents have significantly decreased the gastric MPO levels withanolide A at the dose of 20µg/ml/kg have considerably reduced the gastric MPO levels 5±0.04 U/g tissue among all the other phytoconstituents, whereas Bacoside A and Shatavarin IV showed 5.19±0.10 U/g tissue and 5±0.08 U/g tissue correspondingly Table 2.

Table 2: Phytoconstituents impact on gastric MPO

Group	Dose	MPO (U/g tissue)
Control (0.5%CMC)	5ml/kg	2.27±0.04
Ethanol	5ml/kg	8.14±1.44
Ethanol + Surlafate	5ml/kg +100mg/kg	4.25±0.13***
Ethanol +Bacoside A	5ml/kg +200µg/ml/kg	5.19±0.10*
Ethanol + Withanolide A	5ml/kg +20µg/ml/kg	5±0.04*
Ethanol + Shatavarin IV	5ml/kg +200µg/ml/kg	5±0.08*

Values are interpreted as (Mean ± S.E.M.), n=6,* p<0.05, * ** p<0.001 relative to the control group. (Statistically evaluated by a one-way variance analysis (ANOVA) accompanied by a t-test by Dunnet.) Statistically significant results were rendered at P<0.05.

Effect in inflammatory cytokines - TNF-ALPHA

Marked elevation of the TNF-α in the ethanol treated group 3931.2±68.29 pg /g tissue when compared to the control group 994.26 ± 12.97pg/g tissue. The standard drug sucralfate at the dose of 100mg/kg significantly decreased the inflammatory cytokine TNF- α levels to 1134.98±22.71 pg/g tissue. Meanwhile the phytoconstituents decreased the TNF-α as follows Bacoside A -1837.23±23.75 pg/g tissue, Shatavarin IV - 1615.6±24.9 pg/g tissue and Withanolide A showed best ulcer healing activity as 1534±32.21 pg/g tissue Fig. 1.

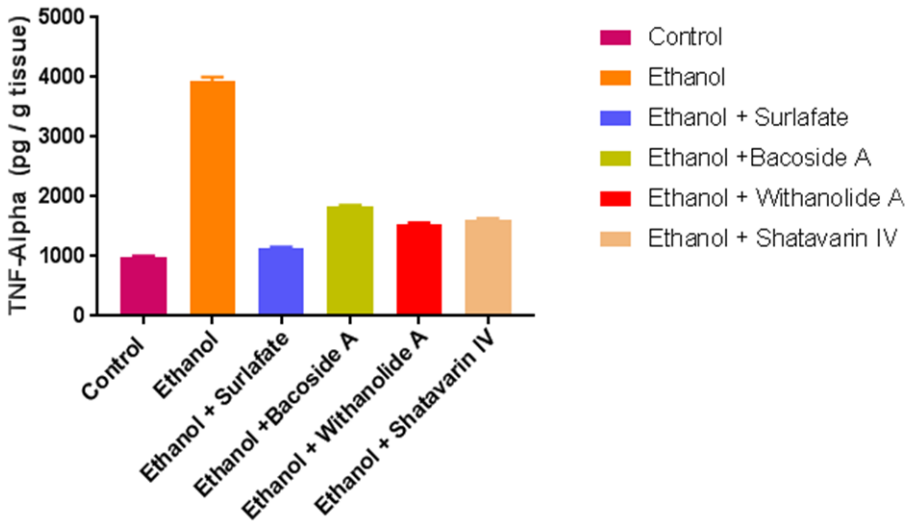

Fig. 1: Phytoconstituents impact on inflammatory cytokines – TNF ALPHA.

Effect in inflammatory cytokines – IL-10

Interleukin-10 levels were abridged in the ethanol treated group as 658.18±5.09 pg /g tissue, treatment with the standard drug has engorged the IL-10 levels to 1234.11±8.71 pg /g tissue. the phytoconstituents Bacoside A showed greater raise in the IL-10 levels as 1127.91±10.92 pg /g tissue, Withanolide A and Shatavarin IV also showed significant increase in the IL-10 levels 1045.51±33.66 pg /g tissue, 999.9±2.606 pg /g tissue respectively Fig. 2.

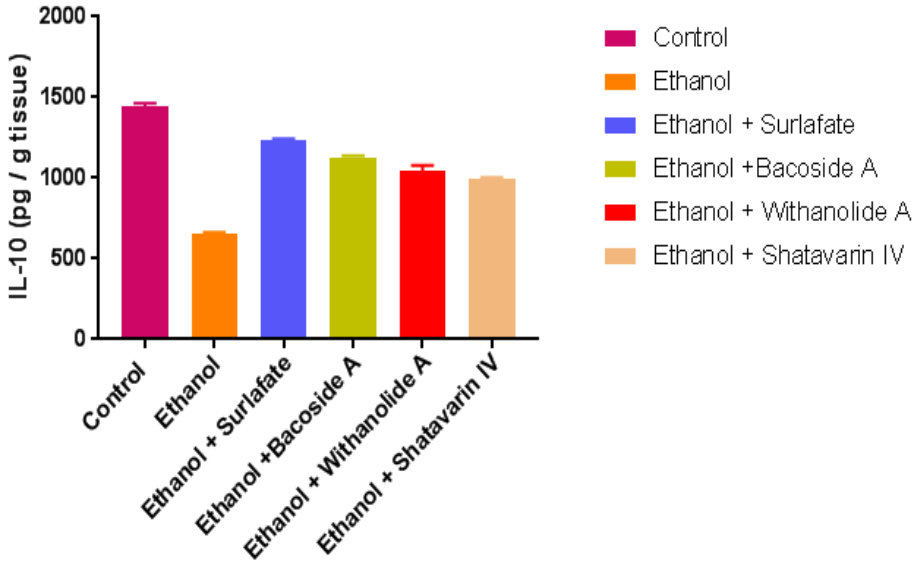

Fig. 2: Phytoconstituents impact on inflammatory cytokines – IL-10.

Caspase-3 activity

Caspase-3 levels were 0.97±0.01 fold change in the control group, the levels were abnormally increased in the ethanol group to 2.32±0.01 fold change. Standard drug treatment decreased the levels to 1.31±0.01 fold change. The phytoconstituent Withanolide A showed the superior activity among all the phytoconstituents as 1.57±0.01 fold change. The Bacoside A and Shatavarin IV also had shown significant increase in Caspase-3 levels 1.68±0.02 fold change, 1.61±0.00 fold change respectively Fig. 3.

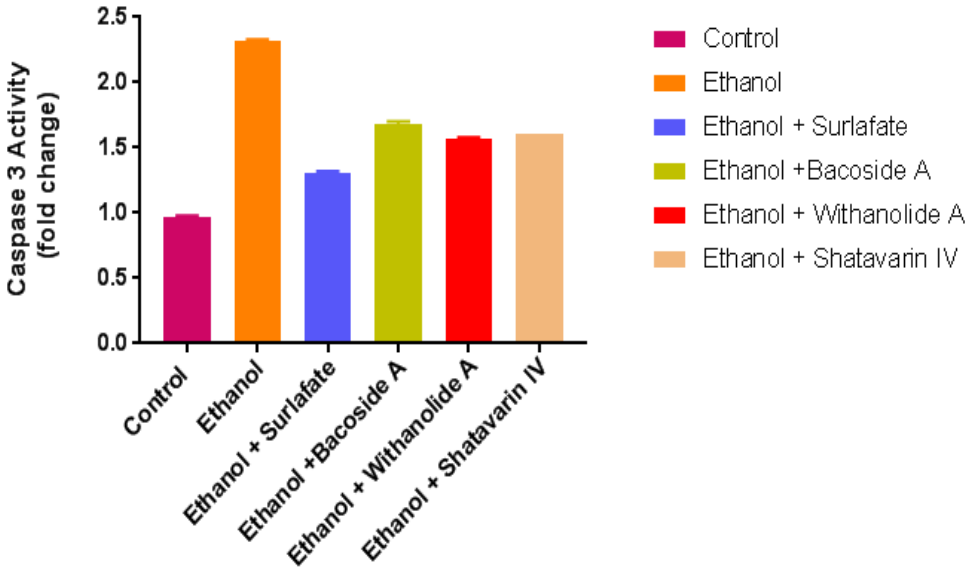

Fig. 3: Phytoconstituents impact on caspase- 3 activity

EFFECT ON ANTIOXIDANT PARAMETERS

Effect on MDA level

Ethanol treatment have triggered the oxidative stress by increasing the MDA level in ethanol treated group to 39.13±0.80 n mol/ g tissue, standard drug treatment diminished the oxidative stress by lowering the MDA levels to 22.21±0.49 n mol/ g tissue, the treatment with different phytoconstituents also had same effect as like that of the standard drugs contributing to reduction of oxidative stress and ulcer protection, Bacoside A had reduced the MDA levels significantly to 25.36±0.27 n mol/ g tissue Table 3.

Table 3: Phytoconstituents impact on gastric MDA.

Group	Dose	MDA (n mol/ g tissue)
Control (0.5%CMC)	5ml/kg	18.2±0.21
Ethanol	5ml/kg	39.13±0.80
Ethanol + Surlafate	5ml/kg +100mg/kg	22.21±0.49***
Ethanol +Bacoside A	5ml/kg +200µg/ml/kg	25.36±0.27***
Ethanol + Withanolide A	5ml/kg +20µg/ml/kg	28.8±0.31*
Ethanol + Shatavarin IV	5ml/kg +200µg/ml/kg	26.16±0.22*

Values are interpreted as (Mean ± S.E.M.), n=6,* p<0.05, *** p<0.001 relative to the control group.(Statistically evaluated by a one-way variance analysis (ANOVA) accompanied by a t-test by Dunnet.) Statistically significant results were rendered at P<0.05.

Effect on GSH level

The GSH level were decreased with ethanol treatment whereas the levels augmented to 1524.2±21.49 n mol/ g tissue with the treatment of standard drug, even though all the phytoconstituents showed significant activity, the phytoconstituent Bacoside A showed peak level of GSH 1378.53±10.65 n mol/ g tissue when compared to others Table 4.

Table 4: Effect of various phytoconstituents on gastric GSH

Group	**Dose**	**GSH (n mol/ g tissue)**
Control (0.5%CMC)	5ml/kg	1721±5.06
Ethanol	5ml/kg	457.86±4.25
Ethanol + Surlafate	5ml/kg +100mg/kg	1524.2±21.49***
Ethanol +Bacoside A	5ml/kg +200µg/ml/kg	1378.53±10.65*
Ethanol + Withanolide A	5ml/kg +20µg/ml/kg	1332.28±7.37*
Ethanol + Shatavarin IV	5ml/kg +200µg/ml/kg	1371.92±12.24*

Values are interpreted as (Mean ± S.E.M.), n=6,* p<0.05, * ** p<0.001 relative to the control group. (Statistically evaluated by a one-way variance analysis (ANOVA) accompanied by a t-test by Dunnet.) Statistically significant results were rendered at P<0.05.

Effect on GPX level

GPX Level were decreased with the treatment of ethanol to2.33±0.16 U/mg Protein, treatment with the standard drug have increased the CPx level to 7.06±0.13 U/mg Protein. Phtoconstituents also notably increase the GPx level as like that of the standard drug. Bacoside A- 6.01±0.06 U/mg Protein, Withanolide A 5.91±0.04U/mg Protein and Shatavarin IV 6.16±0.11 U/mg Protein. The Withanolide A showed preeminent activity Fig. 4.

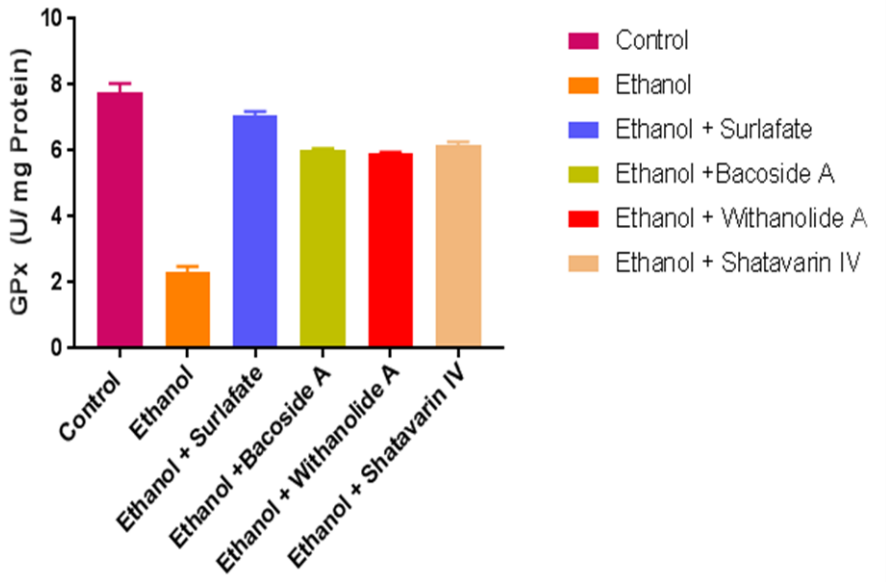

Fig. 4: Phytoconstituents impact on gastric GPX

Effect on TAC level

TCA level were decreased by the treatment of ethanol to 28.06±0.48 µ moltrolox equivalent/ g tissue, Standard drug Sucralfate at the dose of 100mg/kg have amplified the TCA levels to 43.33±0.35 µ moltrolox equivalent/ g tissue, a significant increase in the TCA level in the phytoconstituent treated groups, Shatavarin IV showed marked raise in the TCA level Fig. 5.

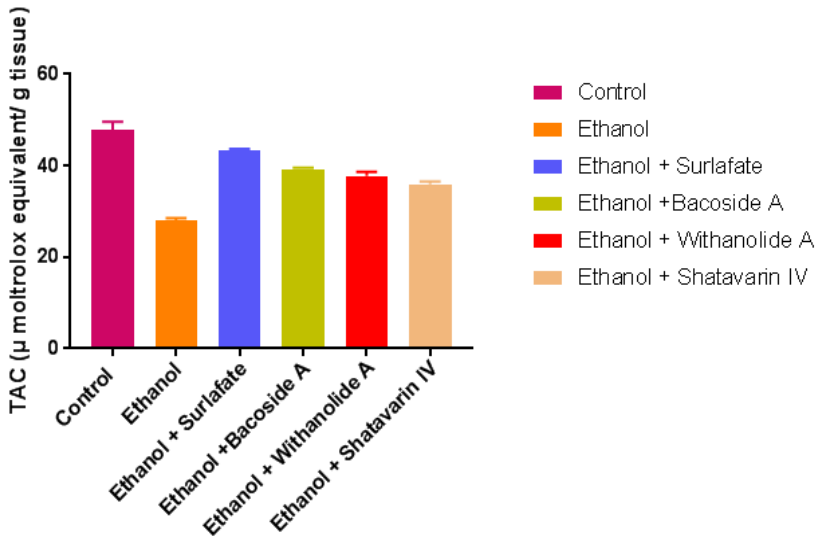

Fig. 5: Phytoconstituents impact on gastric TAC

DISCUSSION

The phytoconstituents Bacoside A, Withanolide A and Shatavarin IV were obtained from the herbal plants Bacopamonniera, Withania somnifera and Asparagus racemosus have important pharmacological effects, and there has been a lack of

detailed understanding of the capacity of phytoconstituetns. The protective function of the various phytoconstituents was first studied including Bacoside A, Withanolide A and Shatavarin IV.

In ethanol-induced gastric injury process, ethanol induces gastrointestinal damage by direct effects including destruction of the cell membrane of mucus, cytotoxic and dehydration effects with the consequent dissemination of the inflammatory cascade [31]. Meanwhile, alcohol induces apparent detrimental effects by recruiting leukocytes that trigger inflammatory responses, oxidative stress and apoptosis. NF-κB plays a crucial role in mediating the interplay between these events [31, 26]. Specific phytoconstituetns delivered significant defense against gastric ulcer induced by ethanol, primarily through the suppression of NF-κB. This was achieved either directly by inhibiting downstream targets such as the proinflammatory TNF-α or indirectly by battling ROS through the antioxidant properties of various phytoconstituetns. Furthermore, the phytoconstituetns' anti-apoptotic and cytoprotective effects also mediated the protection against ethanol insult. Such gastro-protective acts were essentially similar to those performed by the reference sucralfate, which signified the possible use of phytoconstituetns to relieve ethanol-provoked gastric lesions.

Ethanol-induced stomach injury is a primary experimental model widely used for preclinical evaluation of agents with potential anti-ulcer activity since ethanol was found to be a leading cause of stomach ulcer in humans [32-34]. Alcohol has been reported to cause hemorrhagic gastric lesions characterized by mucosal friability, cell exfoliation, severe submucosal edema, and infiltration of inflammatory cells [31, 35]. Additionally, ethanol results in blood flow stasis and destruction of gastric micro-vessels; events that cause hemorrhage and necrotic stomach damage [31, 36].

Gastric mucosal injury is provoked by PMN cell invasion as indicated by MPO activity which also produces hypochlorus acid which causes acute inflammation and stomach damage [37]. In the current research, phytoconstituents attenuated gastric histopathological aberrations and the influx of leukocytes as evidenced by suppression of MPO activity signifying their possible anti-ulcer acts. Such findings are consistent with prior studies [31, 36]. The abolition of neutrophil infiltration has been considered a crucial anti-inflammatory mechanism which protects effective anti-ulcer agents against gastric ulcerative lesions [38]. Such favorable behavior are probably mediated by the observed phytoconstituents inhibition of TNF-α and oxidative stress as they induce the expression of several adhesion molecules, including ICAM-1, which increase the leukocyte invasion of injured gastric mucosa [31, 39].

Current data revealed that ingestion of ethanol upregulated the inflammatory response as evidenced by increased gastric proinflammatory TNF-α and increased protein expression of activated NF-κBp65 in rats. This has been accompanied by a decrease in the anti-inflammatoryIL-10. These findings conform to previous reports [36, 38, 39]. TNF-α has been closely linked to gastric inflammation via the activation and recruitment of immune cells, the production of other proinflammatory cytokines and NF-κBupregulation [39, 40].

Also, TNF-α suppresses gastric microcirculation around ulcerated mucosa, slowing its healing42. Conversely, MHC class II antigen presentation and subsequent release of pro-inflammatory cytokines was reported to be down-regulated by IL-10 and thus its decreased levels exacerbate gastric lesions [32, 36]. In the same sense, NF-κBis a transcription factor that mediates critical inflammatory events in ethanol-induced stomach injury including the expression of several downstream proinflammatory targets such as TNF-α, chemokines such as IL-8 and adhesion molecules [26, 36, 42, 43]. In the inactive state, NF-κB, a heterodimer of the Rel protein family p65 and p50 subunits, is located in cell cytosol and is kept inactive by binding to the inhibitory protein IκBα. The IκBα undergoes phosphorylation and subsequent proteasomal degradation after exposure to stress signals such as ROS and inflammatory cytokins. As a result, activated NF-κB is released and then translocates to the nucleus to interact with DNA response elements to mediate target inflammatory genes transcription [44]. Therefore, the NF-κB p65 subunit was generally considered a marker for the activation of NF-κB [45, 46]. Notably, several inflammatory mediators have been known to trigger the NF-κB, such as TNF-α and IL-1β, which generate ROS and thus involve ROS as a specific NF-Kbactivator [43].

Ironically, with an enhancement of the anti-inflammatoryIL-10 levels, all hytoconstituents

suppressed TNF-α and NF-κB p65. It can be concluded that the inhibition of NF-κB is a chief mechanism for the suppression of gastric inflammatory response by phytoconstituents since the Expression of proinflammatory cytokines TNF-α is mainly regulated by the transcription of NF-κB [36, 40]. The inhibition of NF-κB p65 expression along with its downstream targets such as TNF-α was thus seen as a successful strategy for managing gastric injury [36, 47]. Meanwhile, the above findings confirmed the histopathological results that identified the dwindling of immune inflammatory cell infiltration, edema and hemorrhage in phytoconstituents. The research work shows the defensive actions of ethanol-induced gastric damage phytoconstituents, which were partially mediated by their multi-pronged immune modulatory and anti-inflammatory behavior.

The present findings also identified an in vivo activation of apoptosis in gastric tissues treated with ethanol, as demonstrated by caspase-3 up regulation. In ethanol-induced gastric mucosal injury, enhanced apoptotic death of gastric epithelial cells has been inferred in part [48, 49]. Inflammatory signals along with oxidative stress have been documented to cause the expression by apoptosis of several genes responsible for cell death [27].

The current data showed that phytoconstituents were inhibited caspase-3, implying attenuation of apoptosis of the gastric mucosa. Mucosal apoptosis attenuation can be due to the observed lipid peroxidation suppression and TNF-α because excessive exposure of gastric mucosa to ROS and TNF-α has been documented to increase gastric epithelial apoptosis [50].

Ethanol has been reported to decrease gastric mucosal microcirculation resulting in hypoxia, ROS generation and lipid peroxidation associated [39]. In this study, ethanol administration instigated gastric oxidative stress and increased the lipid peroxide levels in a neutrophil activation-driven process. It also depleted the antioxidant defenses in the gastric GSH, GPx and TAC that scavenge free radicals and prevent their detrimental effects [31]. Depleting GSH, that mostly plays a key role in the brawl against oxidative stress and cell damage, makes gastric tissues more susceptible to oxidative injury [31, 39]. Furthermore, suppression of GPx, an essential antioxidant protection that protects against oxidative aberrations, exacerbates gastrointestinal injury caused by ethanol.

Current research results showed that phytoconstituents combated oxidative stress and improved the antioxidant status in animals with ethanol gastritis as shown by decreasing MDA levels in addition to increasing GSH, GPx and TAC rates. The abrogation of neutrophil infiltration found in the current study can be due to an alternative possible reason for the antioxidant effects of phytoconstituents. In addition, ROS scavenging has been considered as one of the mechanisms involved in ulcer cure [50]. Phytoconstituents' antioxidant activities may play a role in attenuating gastric inflammatory response through inhibition of the redox-sensitive NF-κB cascade [26]. Preserving GSH, GPx, and TAC defenses means the role of phytoconstituents in boosting mucosal antioxidant defenses Together, the antioxidant behavior observed probably contribute to protecting phytoconstituents from mucosal injury.

CONCLUSION

By various pathways, the phytoconsistencies Bacoside A, Withanolide A and Shatavarin IV showed effective protective action against ulcer and ulcer healing action. More analysis will aid with minimal dose and optimum exercise without any side effects in the development of new anti-ulcer medication.

REFERENCES

[1]. Bharathi DP, Jegad E and Kavimani S: Antiulcer activity of aqueous extract of fruits of Momordicacymbalaria in Wistar rats. Pharmacognosy Res 2(1), 2010, 58-61.

[2]. Panneerselvam S and Arumugam G: A biochemical study on the gastroprotective effect of hydroalcoholic extract of Andrographispaniculata in rats. Indian Journal of Pharmacology. 43, 2011, 402-408.

[3]. Arumugam S, Selvaraj SV, Velayutham S, Natesan SK and Palaniswamy K: Evaluation of anti-ulcer activity of Samaneasamanmerr bark on ethanol and stress induced gastric lesions in albino rats. Indian Journal of Pharmacology 43, 2011, 586-590.

[4]. Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly CK, Chakraborty T, Bhattacharya K and Banerjee RK:Gastroprotective effect of neem bark extract possible involvement of H+K+ATPase inhibition and scavenging of hydroxyl radical. Life Sciences 71, 2002, 2845-2865.
[5]. Majumdar S, Base A, Paul P, Halder M and Jha S: Bacosides and neuroprotection. In, Ramawat KG, and Mérillon J-M (Eds.). Natural Products. Springer Berlin Heidelberg, Germany, 2013, 3639-3660.
[6]. Deepak M, Sangli G.K, Arun PC and Amit A: Quantitative determination of the major saponin mixture bacosideA in Bacopamonnieri by HPLC. Phytochemical Analysis 16(1), 2005, 24–29.
[7]. Bacoside A, mixture of Bacoside A3, Bacopaside II, Bacopaside X and Bacopasaponin C. Sigma-Aldrich.com Available from https://www.sigmaaldrich.com/catalog/product/sial/76091?lang=en&region=IN.
[8]. Thomas RB, Joy S, Ajayan MS and Paulose CS: Neuroprotective Potential of Bacopamonnieri and Bacosidea against dopamine receptor dysfunction in the cerebral cortex of neonatal hypoglycaemic Rats. Cellular and Molecular Neurobiology 33(8), 2013, 1065–1074.
[9]. Russo and Borrelli: Bacopamonniera, a reputed nootropic plant: an overview. Phytomedicine 12; 4: 305–317.
[10]. Aguiar B: Neuropharmacological Review of the Nootropic Herb Bacopamonnieri. Rejuvenation Research 2013, 1431.
[11]. Bhattacharya S, Bhattacharya A, Kumar A and Ghosal S: Antioxidant activity of Bacopamonniera in rat frontal cortex, striatum and hippocampus. Phytotheraphy Research 14(3), 2000, 174–179.
[12]. Anbarasi K, Vani G, Balakrishna K and Devi: Effect of bacosidea on brain antioxidant status in cigarette smoke exposed rats". Life Sci78(12), 2006, 1378–1384.
[13]. Anbaras K, Sabitha K and Devi CS: Lactate dehydrogenase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of bacoside A. Environmental Toxicology and Pharmacology 20(2), 2005, 345–350.
[14]. Vohora D, Pal SN and Pillai KK: Protection from phenytoin-induced cognitive deficit by Bacopamonniera, a reputed Indian nootropic plant. Journal of Ethnopharmacology 71(3), 2000, 383–390.
[15]. Hota SK, Barhwal K, Baitharu I, Prasad D, Singh SB and Ilavazhagan G: Bacopamonniera leaf extract ameliorates hypobaric hypoxia induced spatial memory impairment. Neurobiology of Disease 34, 2009, 23–39.
[16]. Singh R, Rachumallu R, Bhateria M, Panduri J and Bhatta RS: In vitro effects of standardized extract of Bacopamonniera and its five individual active constituents on human P-glycoprotein activity. Xenobiotica 45(8), 2015, 741-749.
[17]. Rauf K, Subhan F, Abbas M, Ali SM, Ali G, Ashfaq M and Abbas G: Inhibitory effect of bacopasides on spontaneous morphine withdrawal induced depression in mice. Phytotheraphy Research 28(6), 2015, 937-939.
[18]. Muhammad Shahid, FazalSubhan, IhsanUllah, Gowhar Ali, JavaidAlam and Rehmat Shah: Beneficial effects of Bacopamonnieri extract on opioid induced toxicity. Heliyon 2(2), 2016, 68.
[19]. Glotter E: Withanolides and related ergostane-type steroids. Natural Product Reports 8(4), 1991, 415.
[20]. Kurapati K.R.V, Samikkannu T and Atluri V.S: β-Amyloid1-42, HIV-1Bα-L (clade B) infection and drugs of abuse induced degeneration in human neuronal cells and protective effects of ashwagandha (Withaniasomnifera) and its constituent Withanolide A. PLoS One 9(11), 2014, 1-23.
[21]. IswarBaitharu, Vishal Jain, Satya Narayan Deep, SabitaShroff, Jayanta Kumar Sahu, Pradeep Kumar Naik and GovindasamyIlavazhagan:Withanolide A Prevents Neurodegeneration by Modulating Hippocampal Glutathione Biosynthesis during Hypoxia. PLoS ONE 9(10), 2014, e105311.
[22]. Mitra SK, Prakash NS and Sundaram R: Shatavarins (containing Shatavarin IV) with anticancer activity from the roots of Asparagus racemosus. Indian Journal of Pharmacology 44, 2012, 732-736.
[23]. Shankar K, Mitra S, Prakash and Ramachandran S: Anticancer activity from the roots of Asparagus racemosus. Indian Journal of Pharmacology 44(6), 2012, 732–736.
[24]. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi M: Abdualkader AM, Hadi HA and Ali H: In vivo antioxidant and antiulcer activity of Parkiaspeciosaethanolic leaf extract against ethanol-induced gastric ulcer in rats. PLoS 8(5), 2013, 64751.
[25]. Park S, Oh TY, Kim YS, Sim H, Park SJ, Jang EJ, Park JS, Baik HW and Hahm KB: Artemisia asiatica extracts protect against ethanol-induced injury in gastric mucosa of rats. Journal of Gastroenterology Hepatology 23(6), 2008, 976–984.

[26]. Liu Y, Tian X, Gou L, Fu X, Li S, Lan N and Yin X: Protective effect of l-citrulline against ethanol-induced gastric ulcer in rats. Environmental Toxicology and Pharmacology 34(2), 2012, 280–287.
[27]. Abdelwahab SI: Protective mechanism of gallic acid and its novel derivative against ethanol-induced gastric ulcerogenesis: Involvement of immunomodulation markers, Hsp70 and Bcl-2-associated X protein. International Journal of Immuno pharmacology 16(2), 2013, 296–305.
[28]. Buege JA and Aust SD: Microsomal lipid peroxidation. Methods in Enzymology 52, 1978, 302–310.
[29]. Beutler E, Duron O and Kelly BM: Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical medicine 61, 1963, 882–888.
[30]. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193(1), 1951, 265–275.
[31]. Sangiovanni E, Vrhovsek U, Rossoni G, Colombo E, Brunelli C, Brembati L Trivulzio S, Gasperotti M, Mattivi F, Bosisio E and Dell'Agli M: Ellagitannins from Rubus berries for the control of gastric inflammation: in vitro and in vivo studies. PLoS One 8(8), 2013, 71762.
[32]. Salga MS, Ali HM, Abdulla MA and Abdelwahab SI: Gastroprotective activity and mechanism of novel dichlorido- zinc (II)-4- (2-(5-methoxy benzyl ideneamino) ethyl) piperazin-1-iumphenolat e complex on ethanol- induced gastric ulceration. Chemico- Biological Interaction 195(2), 2012, 144–153.
[33]. Santos FA and Rao VS: 1, 8-cineol, a food flavoring agent, prevents ethanol-induced gastric injury in rats. Digestive Disease and Sciences 46(2), 2001, 331–337.
[34]. Guslandi M: Effects of ethanol on the gastric mucosa. Digestive Diseases 5(1), 1987, 21–32.
[35]. Li W, Huang H, Niu X, Fan T, Mu Q and Li H: Protective effect of tetrahydrocoptisine against ethanol induced gastric ulcer in mice. Toxicology and Applied Pharmacology 272(1), 2013, 21–29.
[36]. Wallace JL, Keenan CM and Granger DN: Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. American Journal of Physiology 259, 1990, 462–467.
[37]. Mei X, Xu D, Xu S and Zheng Y: Novel role of Zn (II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers. Chemico-Biological Interaction, 197(1), 2012, 31–39.
[38]. Sacanella E, Estruch R, Badia E, Fernandez-Sola J, Nicolas JM and Urbano-Marquez A: Chronic alcohol consumption increases serum levels of circulating endothelial cell/leucocyte adhesion molecules E-selectin and ICAM-1. Alcohol 34(5), 1999, 678–684.
[39]. Yadav SK, Adhikary B, Chand S, Maity B, Bandyopadhyay SK and Chattopadhyay S: Molecular mechanism of indomethacin-induced gastropathy. Free Radical Biology and Medicine 52(7), 20121, 1175–1187.
[40]. Hasgul R, Uysal S, Haltas H, Akyol S, Yuksel Y, Gurel A and Armutcu F: Protective effects of Ankaferd blood stopper on aspirin-induced oxidative mucosal damage in a rat model of gastric injury. Toxicology and Industrial Health 30(10), 2010, 888–895.
[41]. Ghosh S, May MJ and Kopp EB: NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annual Review of Immunology 16, 1998, 225–260.
[42]. Handa O, Naito Y, Takagi T, Shimozawa M, Kokura S, Yoshida N, Matsui H, Cepinskas G, Kvietys PR and Yoshikawa T: Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappaB. Journalof Pharmacology and Experimental Therapeutics 309(2), 2004, 670–676.
[43]. Chen F, Castranova V and Shi X: New insights into the role of nuclear factor-kappa B in cell growth regulation. American Journal of Pathology 159(2), 2001, 387–397.
[44]. Arab HH, Al-Shorbagy MY, Abdallah DM and Nassar NN: Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. PLoS One 9(5), 2014, 97193.
[45]. Khan AQ, Khan R, Rehman MU, Lateef, Tahir M, Ali F,and Sultana S: Soy isoflavones inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cutaneous inflammation via modulation of COX-2 and NF-kappaB in Swiss albino mice. Toxicology 302(2–3), 20121, 266–274.
[46]. Tahir M, Rehman MU, Lateef, Khan R; Khan AQ; Qamar W Ali F, O'Hamiza O and Sultana S. Diosmin protects against ethanol induced hepatic injury via alleviation of inflammation and regulation of TNF-alpha

and NF-kappaB activation. Alcohol 47(2), 20132, 131–139.

[47]. Yamamoto S, Watabe K; Araki H; Kamada Y; Kato M; Kizu T Kiso S, Tsutsui S, Tsujii M, Kihara S, Funahashi T, Shimomura I, Hayashi N, Takehara T: Protective role of adiponectin against ethanol-induced gastric injury in mice. American Journal of Physiology: Gastrointestinal and Liver Physiology 302(8), 2012, G773–780.

[48]. Ye HH, Wu KJ, Fei SJ, Zhang XW, Liu HX, Zhang JL, and Zhang YM:Propofol participates in gastric mucosal protection through inhibiting the toll-like receptor-4/nuclear factor kappa-B signaling pathway. Clinics and Research in Hepatology and Gastroenterology 37(1), 2013, 3–15.

[49]. Al Moutaery M, Al Rayes H, Al Swailam R, Elfaki I, Khan HA, Arshaduddin M and Tariq M: Protective effect of a cysteine prodrug and antioxidant, L-2-oxothiazolidine-4-carboxylate, against ethanol-induced gastric lesions in rats. Experimental Toxicology and Pathology 64(3), 2012, 233–237.

[50]. Szabo S, Nagy L and Plebani M: Glutathione, protein sulfhydryls and cysteine proteases in gastric mucosal injury and protection. ClinicaChimicaacta, 206(1–2), 1992, 95–105.