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## RESEARCH ARTICLE

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### STUDIES ON THE IN-VITRO ANTIOXIDANT PROPERTIES OF A POLYHERBAL FORMULATION: LIV01-SSA-23

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#### Abstract

**Background:** The newly developed herbal Formulation (LIV01-SSA-23), that has a combination of multiple medicinal herbs such as the powders of *Solanum nigrum*, *Holarrhena antidysentrica*, *Tephrosia purpurea*, *Andrographis paniculata*, *Phyllanthus niruri*, *Tinospora cordifolia*, *Terminalia chebula*, *Asteracantha longifolia*, *Alstonia scholaris*, *Berberis aristata*, *Chichorium intybus*, *Picrorhiza kurroa*, was tested for its antioxidant and free radical scavenging activity in vitro. This PHF has been traditionally used by the ayurvedic practitioners in India, for the treatment of various liver complications and inflammatory disorders.

**Methodology:** The developed formulation was tested for total reducing power and in vitro antioxidant activity by ABTS assay, Superoxide anion scavenging activity assay and Lipid per-oxidation assay.

**Conclusion:** In conclusion, we strongly suggest that Polyherbal formulation are source of potential antioxidant for radical scavenging. The highly positive correlation of anti-radical scavenging activity and total polyphenolic content in Polyherbal compounds indicates that polyphenols are important components which could be used for the free radical scavenging activity. Further study is needed for isolation and characterization of the active moiety responsible for biological activity and to treat in various stress condition.

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#### Introduction:-

It is believed that medicinal plants are a potential source of antioxidants and ROS scavenger molecules [1]. The natural antioxidants tend to be safer and they possess anti-viral, anti-tumor and hepato-protective properties [2,3]. The free radicals and ROS are well-known inducers of cellular and tissue pathogenesis which leads to several chronic human ailments such as rheumatoid arthritis and cancer, as well as the ageing processes. Many anti-inflammatory, digestive, neuroprotective, hepato-protective and anti-ulcer herbal drugs have been recently evaluated and they have been shown to possess anti-oxidant and/or free radical scavenging mechanisms as a part of their activity [4].

Herbal drugs, used in Indian systems of medicine are however claimed to be effective and safe in such ailments. These drugs are considered benign and are particularly valuable for treating chronic diseases that require prolonged therapy. Plant medicines are more often used in combination rather than in a single in order to get maximum benefit from their combined strength. LIV01-SSA-23, a newly developed multi herbal medicine is very useful as a natural hepatoprotective medicine, which compose of a number of Indian medicinal plants such as *Solanum nigrum*, *Holarrhena antidysentrica*, *Tephrosia purpurea*, *Andrographis paniculata*, *Phyllanthus niruri*, *Tinospora cordifolia*, *Terminalia chebula*, *Asteracantha longifolia*, *Alstonia scholaris*, *Berberis aristata*, *Chichorium intybus*, *Picrorhiza kurroa* [5,6]. Animal experiments established that some of plant ingredients of LIV01-SSA-23 have potent antioxidant property but the synergistic action of all these plants still not been evaluated. Generally herbal medicine composed of various plant ingredients makes a synergistic action which produce optimum therapeutic effects with low toxicity and better efficacy. On the other hand our previous work established that LIV01-SSA-23 protect gastric mucosal damage and maintain mucosal lipid profile [7,8]. The objective of this study was to carry out the total antioxidant activity of the developed polyherbal compounds by using different standard tests.

## **Methodology:-**

### **Tested material**

The poly herbal formulation (PHF), LIV01-SSA-23 contains twelve medicinal herbs such as *Solanum nigrum*, *Holarrhena antidysentrica*, *Tephrosia purpurea*, *Andrographis paniculata*, *Phyllanthus niruri*, *Tinospora cordifolia*, *Terminalia chebula*, *Asteracantha longifolia*, *Alstonia scholaris*, *Berberis aristata*, *Chichorium intybus*, *Picrorhiza kurroa*. All the plant materials were procure from the registered supplier.

### **Total reducing potential**

The reducing power of the hydroalcoholic extract of herbal formulation was determined according to the developed method.[9] The 2.5 ml of the solution of extract (100-800 µg/ml) was mixed with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium fericyanide and placed in water bath at 50°C for 20 min. It was then cooled rapidly and 2.5 ml of 10% trichloroacetic acid was added and vortexed. This incubation mixture was centrifuged at 3,000 rpm for 10 min and its 5 ml supernatant was mixed with equal volume of distilled water and 1 ml of 0.1% ferric chloride. It was further incubated at room temperature for 10 min and absorbance was read at 700 nm. The reducing property of test sample was standardized against Quercetin and expressed as difference in O.D. from control, as well as test as 0.1, and expressed as µg/ml. A higher degree of absorbance indicates the stronger reducing power.

### **ABTS assay/Total antioxidant capacity**

ABTS + radical-scavenging activity of polyherbal extracts was determined according to established method [10]. ABTS + radicals were pre-generated by adding 5 ml of a 4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in the dark. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Solution of vitamin C (50 µg/ml) was used as standard. It 50 µl was added to 950 µl of ABTS solution, vortexed for 10 s and after 6 min and then reduction in absorbance was recorded at 734 nm, using distilled water as a blank, on ELICO (SL-150) UV-vis spectrophotometer. Same volume of test solutions of each extract was also taken in similar manner.

### **Superoxide radical-scavenging activity**

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT).[11] In brief, each 3 ml reaction mix-ture contained 0.01 M phosphate buffer (pH 7.8) (PBS), 130 mM methionine, 60 mM riboflavin, 0.5 mM EDTA, NBT (0.75 mM) and 0.5 ml of test sample solution/standard solution. The tubes were kept in front of a fluorescent light and absorbance was taken after 6 min at 560 nm against blank (0.01M PBS). Identical tubes were kept in dark, which served as controls. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control tube with that of test material/standard containing tubes.

### **Lipid per oxidation assay**

A modified thiobarbituric acid-reactive specie (TBARS) assay [12] was used to measure the lipid peroxide.[13] Here, Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA), yielding a pinkish red chromogen with an absorbance maximum at 532 nm. The egg yolk homogenate was prepared in distilled water, (10%, v/v) and 0.1 ml of extract was mixed in a test tube and the volume was made up to 1 ml, by adding distilled water. Finally, 0.05 ml FeSO<sub>4</sub> (0.07 M) was added and incubate it for 30 min, to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with

NaOH) and 1.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of 1-butanol was added to each tube, vortexed thoroughly and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm against blank, where 0.1 ml of distilled water was used in place of the extract.

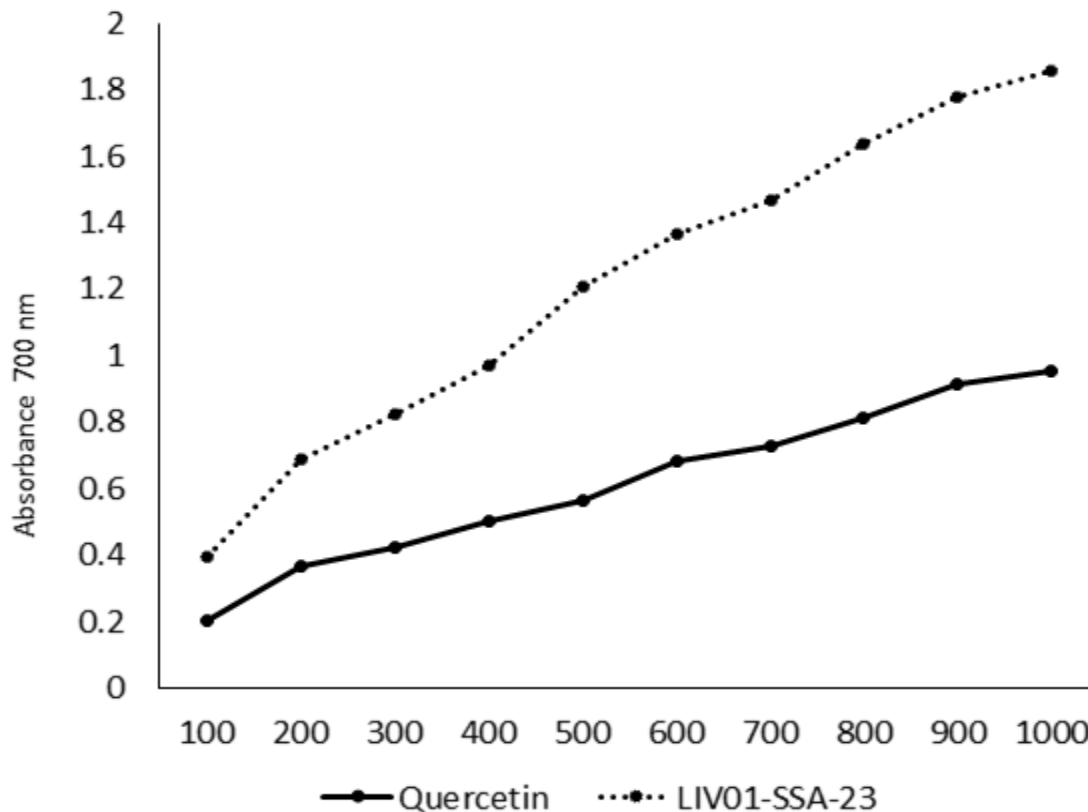
### Statistical analysis

The data were subjected to statistical analysis software SigmaStat version 3.1 for correlation coefficient. The correlation of the data was determined by Pearson's test.  $P < 0.05$  was considered as statistically significant.

### Results:-

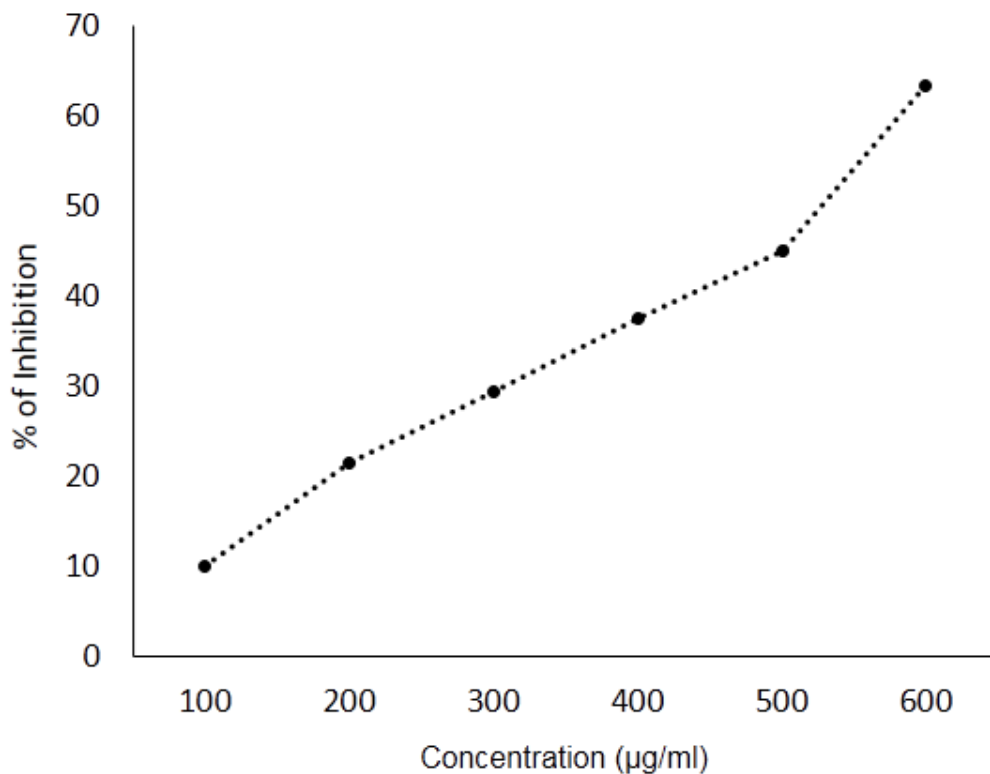
Several concentrations, ranging from 100-1000  $\mu\text{g/ml}$  of the hydroalcoholic extract of LIV01-SSA-23 were tested for their antioxidant activity in different in vitro models. The percentage of inhibition was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models.

Reducing potential of polyherbal combination shown in Figure 1, as a function of concentration dose dependent increase of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  was observed. Amongst different concentration of extracts tested, 100, 200, 400, 600, 800, 1000  $\mu\text{g/ml}$  shows maximum absorption of  $0.906 \pm 0.019$  at 1000  $\mu\text{g/ml}$  compared with standard Quercetin  $0.951 \pm 0.024$ .

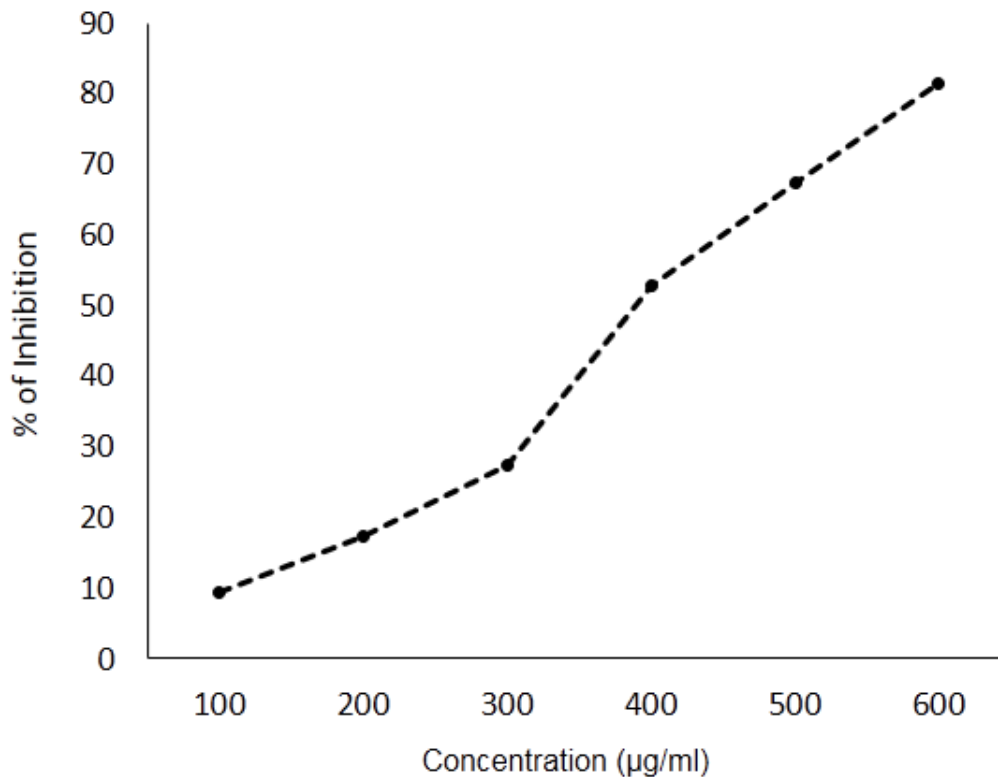


**Figure 1:-** Reducing power of herbal formulation (LIV01-SSA-23) in hydroalcoholic extract and comparison with Quercetin.

Livina in different concentration effectively scavenge the  $\text{ABTS}^+$  radicals showed percentage of inhibition in different concentration of 50, 100, 200, 400, 600, 800, 1000  $\mu\text{g/ml}$  as  $10.04 \pm 1.22$ ,  $21.42 \pm 1.16$ ,  $29.34 \pm 1.02$ ,  $45.02 \pm 1.39$ ,  $63.65 \pm 0.87$ ,  $88.21 \pm 0.81$  (Figure 2). Anti-lipid peroxidation free radicals were scavenged in different concentration of LIV01-SSA-23 in different concentration showed the lipid peroxide radical scavenging activity as  $9.21 \pm 1.84$ ,  $17.29 \pm 1.84$ ,  $31.32 \pm 0.91$ ,  $51.72 \pm 1.28$ ,  $69.28 \pm 1.88$ ,  $81.42 \pm 1.91$  (Figure 3).

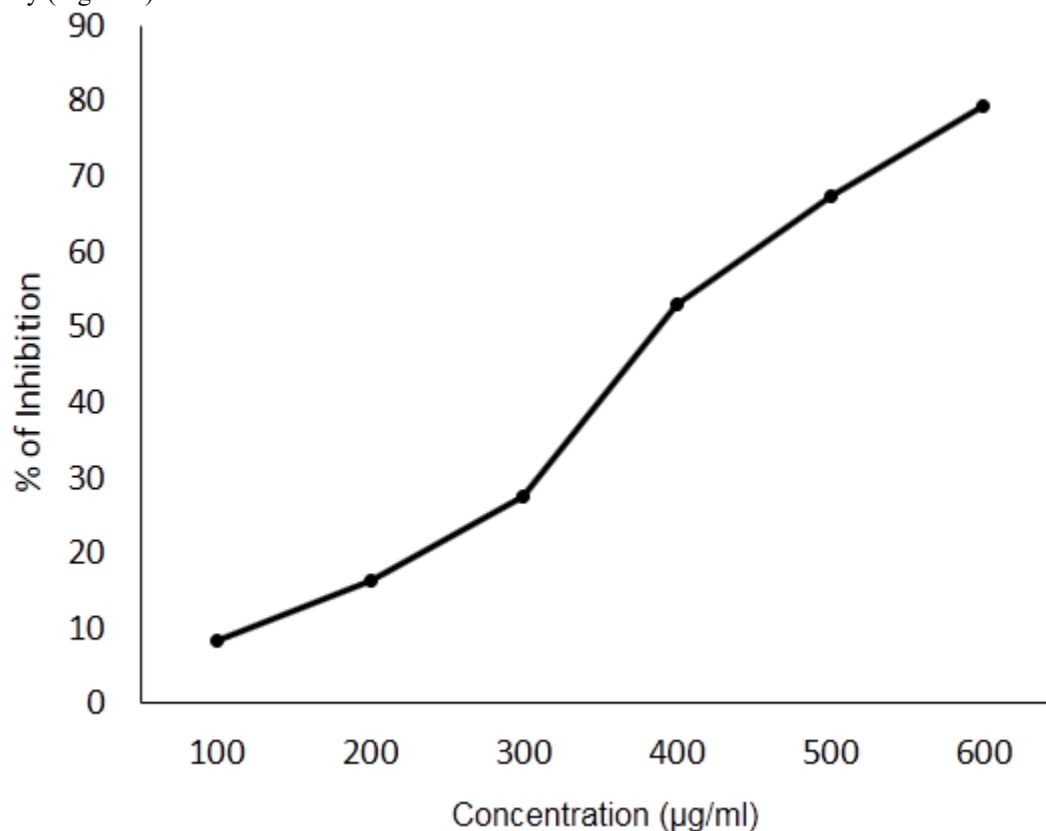


**Figure 2:-** ABTS radical scavenging activity (%). ABTS scavenging activity with different concentrations of herbal formulation (LIV01-SSA-23).



**Figure 3:-** Lipid peroxide scavenging activity (%). Lipid peroxide scavenging activity with different concentrations of herbal formulation (LIV01-SSA-23).

Superoxide free radicals were scavenged in different concentration of LIV01-SSA-23 showed superoxide scavenging activity as (% of inhibition)  $8.29 \pm 1.21$ ,  $16.24 \pm 1.11$ ,  $27.29 \pm 1.80$ ,  $52.81 \pm 0.81$ ,  $67.29 \pm 1.26$  and  $79.29 \pm 1.88$ , respectively (Figure 4).



**Figure 4:-** Superoxide radical scavenging activity (%) with different concentrations of herbal formulation (LIV01-SSA-23). Trapping potential of Copper sulphate.

### Discussion:-

Free radicals are constantly generated within the living system and they can cause severe damage to tissues, cells and biological molecules leading to various diseases.[14] Synthetic drugs are available in the market that can protect our system from oxidative injury but chronic use may lead to various adverse reactions and adverse effects.[15] Currently, many medicinal plant extracts have been shown to be potentially effective as free radical scavengers. Asthma is one among those diseases caused by oxidative stress. The present study aims to evaluate the in vitro antioxidant effect of polyherbal compound namely LIV01-SSA-23. Polyherbal drugs have been extensively used for the management of liver complications since a long time. The efficacy of these drugs is time-tested and clinically proved but validation on scientific parameters is still lacking. Phyto-chemical analysis of the formulation was carried out which showed that the drug has good content of phenolic and flavonoid content. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable.[16,17] Natural phenolics exert their beneficial health effects mainly through their antioxidant activity by decreasing oxygen concentration, intercepting singlet oxygen, preventing 1 chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species, and breaking chains to prevent continued hydrogen abstraction from substances.[18]

ABTS is a blue chromophore produced by the reaction between ABTS and potassium persulfate.[19] Addition of polyherbal extracts to this pre-formed radical cation reduced it to ABTS in a concentration-dependent manner [Figure 2]. These results were compared with those obtained with Quercetin [Figure 1], which indicates that the extract is a potent antioxidant. Similar results were seen with lipid peroxide scavenging by extracts [Figure 3]. Similar trends of scavenging superoxide anion indicate that polyherbal extracts are potent superoxide scavengers [Figure 4]. Superoxide dismutase catalyses the dismutation of the highly reactive superoxide anion to oxygen and hydrogen peroxide.[20]

Superoxide anion is the first reduction product of oxygen.[21] This is measured in terms of inhibition of generation of O<sub>2</sub>. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per oxidation and by many other mechanisms and thus prevent disease.[22]

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid per oxidation by ferrous sulphate takes place either through ferryl-perferryl complex [23] or through ·OH radicals by Fenton reaction [24] thereby initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons viz, the inhibition of ferryl- perferryl complex formation; scavenging of OH or superoxide radicals or by changing the ratio of Fe<sup>3+</sup> /Fe<sup>2+</sup>, reducing the rate of conversions of ferrous to ferric or by chelating of the iron itself. The moderate activity of the extract may probably be due to the rapid and extensive degradation of the antioxidant principles in an ex vivo state.[25]

### Conflict of Interest

We declare that we have no conflict of interest.

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