# *In vivo* anti-oxidant activity of Dhatrinisha churna and Manjisthadi churna in high fat diet induced oxidative stress

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## ABSTRACT

**Background:** Dhatrinisha churna and Manjisthadi churna have been traditionally used in the Ayurvedic system of medicine and by traditional medical practicioners of India to treat hyperlipidemia. **Objective:** To evaluate the anti-oxidant activity of Dhatrinisha churna and Manjisthadi churna in experimental hyperlipidemic rats. **Materials and Methods:** Hyperlipidemia was induced by administrating exogenous cholesterol. Assessment of anti-oxidant status for liver was carried out by assay of various physiological enzymatic (like SOD, catalase, gluatathione peroxidase) and non enzymatic (GSH, lipid peroxidative indices) antioxidant. **Result:** Significant increase in the level of enzymes like SOD, catalase, glutathione peroxidase, GSH and decrease in lipid peroxidase were observed in drug treated animals. **Conclusion:** In conclusion, these observations show that Manjisthadi churna and Dhatrinisha churna exert their protective effect by improving antioxidant status and decreasing the lipid peroxidation, thus establishing them as an effective antioxidant.

**Keywords:** Manjisthadi churna, Dhatrinisha churna, anti-oxidant activity.

## INTRODUCTION

Fat is an important dietary component, which affects both growth and health. It is widely accepted that high level of fat in the diet is detrimental to health. Replacing the traditional cooking fats, considered atherogenic with refined vegetable oils promoted as 'heart friendly' because of their PUFA content, has resulted in increased prevalence of heart disease in India.[1] Current data on dietary fats indicates that it is not just the presence of PUFA but the type of PUFA that is important. The newer heart friendly oils like sunflower oil possess this undesirable PUFA content and thus excess intake of these vegetable oils is actually detrimental to health. Moreover heating of oil is known to alter its nutritional properties especially when it is rich in PUFA. During deep fat frying many volatile and non-volatile products are produced, some of which are toxic depending on the level of intake.[2]

Dhatrinisha churna is an Ayurvedic preparation mentioned in the *Chikisthasthan,* Chapter-II, Slock-8 of Ayurvedic literature *Susrut Samhita* and Chapter-6, Slock-26, 772 of Ayurvedic literature *Charak Samhita*.[3] It consists of a mixture of the fine powder of the dried rhizome of Haridra (*Curcuma longa* Linn.; Zingiberaceae) and dried fruit of Amalaki (*Emblica officinalis* Gaertn. Syn. *Phyllanthus emblica* Linn.; Euphorbiaceae). Manjisthadi churna is an Ayurvedic preparation mentioned in the Chapter *Trutiya Sanskar* of Ayurvedic literature *Bruhad Ausadhiya Shuchi Patra: Therapeutic Index*.[4] It consists of a mixture of the fine powder of the dried stem of Manjistha (*Rubia cordifolia* Linn.; Rubiaceae), dried leaf of Svarnapatri (*Cassia angustifolia* Vahl..; Leguminosae), dried pericarp of fruits of Haritaki (*Terminalia chebula* Retz.; Combretaceae) and dried root of Trivrit (*Operculina turpethum* Linn.; Syn. *Ipomoea turpethum;* Convolvulaceae).

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## MATERIALS AND METHODS

### Plant materials

Individual components of Dhatrinisha churna and Manjisthadi churna were procured from M/s Yucca Enterprise, Mumbai, India, and authenticated by a botanist in comparison with herbarium specimens. The drugs were cleaned, dried and powdered separately and passed through number 40 sieve. All these powders were mixed well in equal proportion uniformly.

### Preparation of extracts

Dhatrinisha churna, Manjisthadi churna and its individual ingredients (1 kg) were extracted exhaustively with distilled water by cold percolation method (3×72 h). The solvent was distilled off over boiling water-bath and the extracts so obtained were dried in a vacuum desiccator till free from moisture.

### Animals

Healthy Wistar rats of either sex weighing 200−250 gm were procured from Experimental Animals Laboratory of the Department of Pharmacology, Baroda College of Pharmacy. They were housed at a room temperature of 25 ± 2°C, relative humidity of 75 ± 5% and 12 h dark-light cycle. They were kept in polypropylene cages with husk renewed every 24 hr provided with standard diet (composite of crude protein: 22.15%, crude oil: 4.11%, crude fiber: 3.34%, Ash- 5.11%, sand silica: 1.15% producing energy of 3620 Kcal/kg) and water *ad libitium*. Animals were free to access water and feed. Experimental protocols had been approved by IAEC.

### Experimental design

Healthy and either sex animals were age matched and grouped according to following, where each group containing 6 animals.[5]

| | |
|---|---|
| Group I (NR) : | Normal animal (Vehicle) |
| Group II (CT) : | Control animal (High fat diet + Vehicle) |
| Group III (DC) : | Dhatrinisha churna extract (DCE) treated animal (High fat diet + DCE) |
| Group IV (TM) : | Haridra extract (TME) treated animal (High fat diet + TME) |
| Group V (AM) : | Amalaki extract (AME) treated animal (High fat diet + AME) |
| Group VI (MC) : | Manjisthadi churna Extract (MCE) treated animal (High fat diet + MCE) |
| Group VII (RB) : | Manjistha extract (RBE) treated animal (High fat diet + RBE) |
| Group VIII (SE) : | Svarnapatri Extract (SEE) treated animal (High fat diet + SEE) |
| Group IX (HR) : | Haritaki Extract (HRE) treated animal (High fat diet + HRE) |
| Group X (TV): | Trivrit Extract (TVE) treated animal (High fat diet + TVE) |

### Estimation of superoxide dismutase

Superoxide dismutase (SOD) was assayed utilising the technique of Kakkar et al.[6] A single unit of enzyme was expressed as 50% inhibition of NBT (nitroblue tetrazolium) reduction/min/mg protein.

### Estimation of catalase

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as µmoles of $H_2O_2$ consumed/min/mg protein as described by Sinha.[7] The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of 2 M $H_2O_2$. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

### Estimation of glutathione peroxidise

Glutathione peroxidase (GPx) activity was measured by the method described by Ellman.[8] Briefly, reaction mixture contained 0.2 ml of 0.4 M phosphate buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of tissue homogenate (homogenised in 0.4 M, phosphate buffer pH 7.0), 0.2 ml glutathione, 0.1 ml of 0.2 mM hydrogen peroxide. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Ellmans reagent.

### Estimation of reduced glutathione

Reduced glutathione (GSH) was determined by the method of Ellman.[8] To the homogenate added 10% TCA, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobis-nitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

### Estimation of lipid peroxidase

Lipid peroxidation as evidenced by the formation of TBARS and HP were measured by the method of Niehaus and Samuelsson[9] and Jiang et al.[10] respectively. In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBATCA-HCl reagent (thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1,000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm.

### Estimation of total protein

The total protein was estimated by total protein and albumin kit (No. 72111) from Qualigens fine chemicals, Worli, Mumbai.

### Preparation of tissue homogenate

Twenty four hours after the last dose was administered, animal fasted overnight were weighed and sacrificed under mild ether anesthesia. After exsanguinations, the liver was removed quickly, washed thoroughly in PBS (phosphate buffer saline), weighed and then homogenized in phosphate buffer (0.1M, pH 7.4). The homogenate was then centrifuged at 15200 rpm at 4°C for 30 min to obtain a clear supernant. The supernant had been used as early as possible for the estimations of tissue parameters.

### Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Values were considered statistically significant when $P \leq 0.05$.

## RESULT AND DISCUSSION

This study analyses the protective role of Dhatrinisha churna and Manjisthadi churna on oxidative stress induced by a high fat diet. Our results showed that there was a significant reduction in oxidative stress in both treatments. Results obtained were shown in Figures 1 to 5.

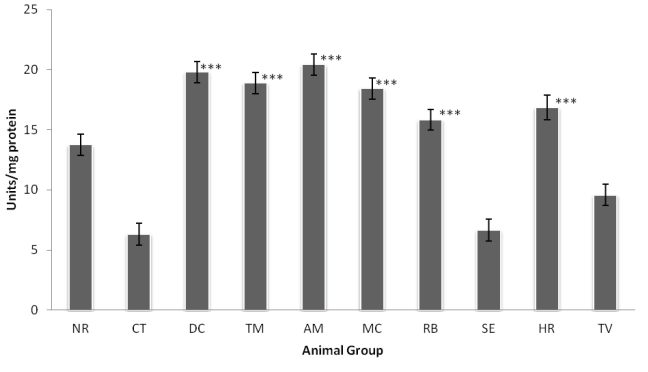


**Figure 1.** Effect of Manjisthadi churna and Dhatrinisha churna on SOD level of Liver tissues of different groups of rats (Units/mg protein).

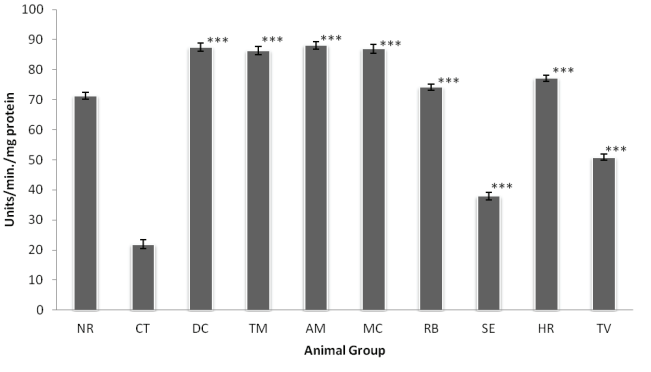


**Figure 2.** Effect of Manjisthadi churna and Dhatrinisha churna on Catalase level of Liver tissues of different groups of rats (Units/mg protein).

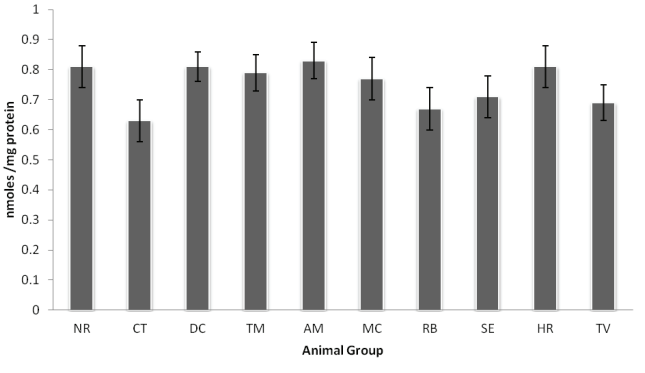


**Figure 3.** Effect of Manjisthadi churna and Dhatrinisha churna on Reduced Glutathione level of Liver tissues of different groups of rats (nmoles/mg protein).

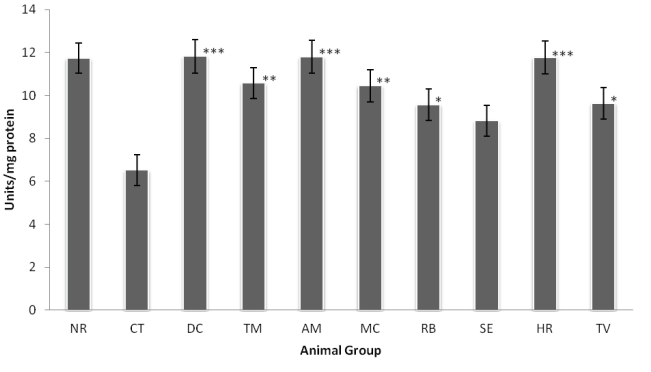


**Figure 4.** Effect of Manjisthadi churna and Dhatrinisha churna on Glutathione Peroxidase level of Liver tissues of different groups of rats (Units/mg protein).

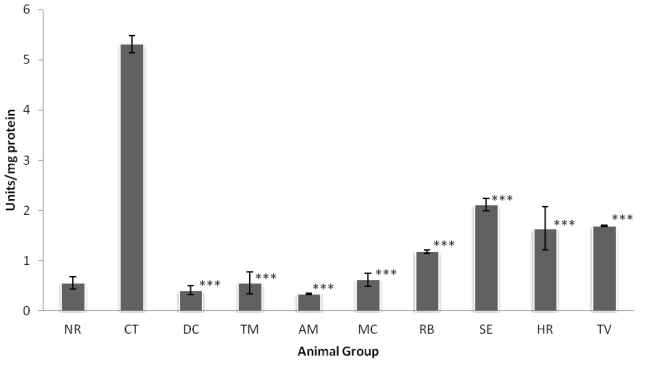


**Figure 5.** Effect of Manjisthadi churna and Dhatrinisha churna on Lipid Peroxidation level of Liver tissues of different groups of rats (Units/mg protein).

The increase in plasma liver markers is a direct reflection of oxidative injury of liver. Moreover, increased intake of high fat diet increases the degree of unsaturation of the biomembrane and makes them more susceptible to lipid peroxidation.[11] Wide utilization of fats which are highly susceptible to oxidation during cooking and frying may alter physiological effects of their PUFA content and generate lipid peroxides that cause membrane damage and increase lipid infiltration and hence make the membrane leaky to liver markers.[12]

Glutathione, an important cellular reductant is involved in protection against free radicals, peroxides and other toxic components.[13] In addition to serving as a strate for glutathione related enzymes, GSH acts as a free radical scavenger, a generator of a α-tocopherol and plays an important role in the maintenance of protein sulfhydryl groups.[14] In the present study, the levels of GSH were decreased significantly in high fat diet ingestion indicating the oxidative stress. GPx has a well-established role in protecting cells against oxidative injury. GPx is non-specific for $H_2O_2$ and lack of this substrate specificity extends a range of substrates from $H_2O_2$ to organic hydroperoxides.[15] Therefore, the excess $H_2O_2$ and lipid peroxides generated during high fat diet ingestion are efficiently scavenged by GPx activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanisms during high fat diet ingestion. Catalase, which acts as preventative antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation.[16] The inhibition of CAT activity is suggestive of enhanced synthesis of $O_2$°- during the ingestion of high fat diet since $O_2$°- is a powerful inhibitor of catalase.[17] SOD catalyses the dismutation of radical anions to $H_2O_2$ and $O_2$.[18] Numerous studies have shown the importance of SOD in protecting cells against oxidative stress.[19] Our study has shown a decrease in SOD activity in tissues during high fat diet ingestion. This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation.[20] The generation of the α-hydroxy ethyl radical may also lead to inactivation of the enzyme.

Administration of Dhatrinisha churna, Manjisthadi churna and its ingredients, decreased the LPO, improved the antioxidant status and thereby prevented the damage to the liver and leakage of enzymes. This is mainly because of the antioxidant sparing action of formulations.

The antioxidant mechanism of anti-oxidant activity may include one or more of the following interactions: scavenging or neutralizing of free radicals,[21] interacting with oxidative cascade and preventing its outcome,[22] oxygen quenching and making it less available for oxidative reaction,[21] inhibition of oxidative enzymes like cytochrome P450[21] and chelating and disarming oxidative properties of metal ions such as iron.[23] Thus in this work Dhatrinisha churna and Manjisthadi churna effectively prevented tissue damage by decreasing the oxidative stress and restoring the antioxidant status. However, the treatment with Dhatrinisha churna was found to be more effective compared to Manjisthadi churna.

## CONCLUSION

Their activities were increased significantly in high lipid diet groups, which were decreased on treatment with Dhatrinisha churna and Manjisthadi churna. However, Dhatrinisha churna and Manjisthadi churna treatment decreased their activity more significantly compared to their individual component.

The levels of SOD, CAT, GPx and GSH were increased significantly in high fat diet group compare to normal group, which were decreased significantly on treatment with Dhatrinisha churna and Manjisthadi churna. The decrease was more significant in Dhatrinisha churna and Manjisthadi churna treated groups compared to its individual ingredients. The levels of non-enzymic antioxidants:GSH and enzymic antioxidants:SOD, CAT and GPX were significantly depleted in high fat diet group which were increased in both Dhatrinisha churna and Manjisthadi churna treatment.

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