ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

COMPARATIVE STUDY OF OXIDATIVE STRESS MARKER MALONDIALDEHYDE ON *DASAMOOLARISHTA* AND ETHANOL TREATED HEALTHY WISTAR ALBINO RATS

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Received on: 20/06/2015 Revised on: 11/07/2015 Accepted on: 20/07/2015

ABSTRACT

Malondialdehyde (MDA) is an end product of lipid peroxidation, involved in several enzymatic and non enzymatic reactions. The present study compares the level of malondialdehyde on self generated alcoholic product *Dasamoolarishta* with the lab grade ethanol treated healthy rats. In this study, 18 *Wistar albino* male rats of the same age, littermates and body weight groups were used. Among these, six of each were grouped into Control (C), *Dasamoolarishta* treated (Test 1) and 6 % Ethanol treated (Test 2) groups. Duration of this study was 30 days. Before starting the experiment, body weight and fasting blood glucose level was checked and verified that the animals were healthy. From the first day onwards the control group was orally treated with tap water (1 ml/kg body weight) and test groups were orally treated with *Dasamoolarishta* (1 ml/kg body weight) and ethanol treated (1 ml/kg body weight). At the end of the administration period, body weight and fasting blood glucose levels were taken and then the animals were sacrificed and the liver tissue was isolated. The Lipid peroxidation status of liver tissue of both control and test groups were analysed. Body weight, fasting blood glucose and lipid peroxidation (Malondialdehyde - MDA) level in liver tissues of both the groups were statistically analysed using ANOVA. *Dasamoolarishta* treated group showed reduced malondialdehyde level than ethanol treated group. It indicates that self generated alcohol in *Dasamoolarishta* does not have any ill effect, thereby showing a protective role in free radical scavenging.

KEYWORDS: *Dasamoolarishta,* Ethanol, Malondialdehyde, Body weight, Liver.

INTRODUCTION

Free radicals are responsible for damage in tissues which leads to several diseases. The toxic or dangerous substances produced in the body either by normal metabolic process or some induced agents, which are atoms or group of atoms with unpaired number of electrons, can be formed when oxygen interacts with certain molecules. Such types of substances are called free radicals. It may be either free radicals or other non-radical reactive derivatives such as hydrogen peroxide, hypochlorous acid, nitrous acid or lipid peroxides. Non radicals are otherwise called oxidants and easily try to produce free radical reactions. These free radical reactions induce tissue injury and leads to abnormal physiological functions of the body.

Malondialdehyde is a highly reactive compound resulting from lipid peroxidation of poly unsaturated fatty acids. It is one of the markers of oxidative stress. This product might be reflected in the changes of tissue antioxidant system and are responsible for damage to tissues which lead to higher prevalence of metabolic syndrome accompanied by obesity, atherosclerosis, diabetes mellitus, liver injury and age related changes. Malondialdehyde may be produced by a number of ways which include synthesis with very dilute reagents, reactions at very low temperatures, break-up of larger molecules, heat, electrical discharges, electrolysis and chemical reactions etc., that take place in our body. Free radicals or other non-radical derivates are formed not only by these reactions but also from external sources like pollution, alcoholism, smoking and medications [1].

Alcohol is a social drug that affects people in different ways. It can cause serious health, personal and social problems. The issue related with alcohol varies according to the type and amount of consumption of alcohol. Alcohol consumption in humans is a serious health issue because liver is the major organ most susceptible to it. Hepatic cirrhosis is one of the major cause of death in chronic alcoholics.

Alcohol consumption is associated with a number of changes in cell function and the oxidant-antioxidant system[2]. The risk of severe liver damage is dependent on the amount of consumption of alcohol. A very small amount of alcohol consumption may reduce the risk of heart disease[3]. So, the present study aims to focus on a moderate dose of ethanol on oxidative stress status of healthy rats when compared with the same dose of *Arishta*.

Alcohol is one of the self generated products of Ayurvedic preparations including *Asavas* and *Arishtas*. *Asavas* and *Arishtas* are very popular *Ayurvedic* medicines. In the past, many traditional health care systems existed in India. *Ayurveda* is the most popular traditional system among them. This system of medicine is based on the principle of balance and counter balance. It comprises of various types of medicines including fermented forms, namely *Arishtas* and *Asavas*[4]. *Asavas* and *Arishtas are* useful for the management of diabetes. "The said composition comprised a therapeutically effective amount of plant extracts, self generated ethanol to the extent of 7 - 12 % v/v and having not more than 1 - 3 % w/w of sugar content. This invention also provides a novel method for the manufacture of herbal composition in liquid oral dosage form containing a limited amount of self generated ethanol"[5]. *Arishtas* are generally a mixture of ingredients such as main drugs which are used in the form of juice or decoctions with sweetening agents like honey, jaggery etc., as the media for alcohol production. Fermentation inducers like *Dhataki* flowers initiate fermentation process by providing the natural microflora and excipient or *Prakshepa dravya* which are added at a later stage of preparation for colour, aroma and medicinal properties[6].

Dasamoolarishta, is commonly used as a general health tonic. According to *Sahasrayoga, Dasamoolarishta* is traditionally a fermented herbal tonic which contains around 70 types of herbs. This health tonic shows remarkable effect on general strength and in providing immunity. In Ayurveda*, Dasamoolarishta* is a unique combination of ten herbs, where *'Dasa'* meaning ten and *'Moola'* meaning root. *Plumbago zeylanica* (*Chitraka*), *Gmelina arborea* (*Gambhari*), *Aegle marmelos* (*Bilwa*), *Stereospermum suaveolens* (*Patala*), *Oroxylum indicum* (*Dunduka*), *Premna mucronata* (*Agnimantha*), *Solanum indicum* (*Brihati*), *Desmodium gangeticum* (*Shalaparni*), *Tribulus terrestris (Gokshura), Tinospora sinensis (Guduchi)* etc*.* are the main ingredients of *Dasamoolarishta*. So, many plant parts (roots, leaves, fruits, seeds and flowers) are used for the preparation of *Dasamoolarishta,* which have medicinal properties. The important ones are *Vitis vinifera* (*Mundiri*)*, Syzygium aromaticum* (*Grampoo*)*, Sassurea Lappa* (*Velutha kottam*)*, Cuminum cyminum* (*Jeerakam*)*, Cinnamomum verum* (*Ilavangam*)*, Phyllanthus emblica* (*Nellikka*)*, Curcuma longa* (*Manjal*) etc., and about 67 types of herbs and spices are used for the preparation of *Dasamoolarishta.* Each of this herbal part has a significant effect in alternative treatment*. Jaggery* is an important ingredient in fermentation because it is only a sugar source. Fermentation is a chemical transformation process, during this, cells of the herbs ruptures exposing its contents to the bacteria and enzymes for transformation. It may be through the yeast source from *Woodfordia fruticosa* flowers [7]. *Woodfordia* flowers are added to regulate the fermentation process and to promote alcohol formation [8]. Fermentation also removes most of the undesirable sugars from plant materials, making the product more bio-available and eliminates side effects. In addition to these, it not only removes contaminants but also lowers the toxicity of some of the toxic components of plants (bound heavy metals and pesticide residues) and therefore, act as a natural cleansing system[9]. Fermented products or otherwise called medicinal wines have several advantages like better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery into the human body sites [10].

Dasamoolarishta is also widely recommended for cardiac disorders, respiratory diseases, asthma and breathing troubles, cough, pneumonia, gastric irritation and anemia. It is also mixed with other *Arishtas* and used for certain diseases. From the above data, *Dasamoolarishta* showed a potent role in health. In the influence of effect of *Dasamoolarishta*, this present study focussed to compare the level of malondialdehyde on *Dasamoolarishta* treated and lab grade ethanol treated rats containing the same percentage of alcohol (6 %) as in *Arishta* treated rats.

MATERIALS AND METHODS:

Animals

A total of 18 *Wistar albino* male rats (Fig.1) were selected for the study. All the test animals weighed between 160-220 grams and approximately 60 days old. The rats were maintained in the animal house of the Department of Life sciences (Reg # 426/ 02/A/CPCSEA). Rats were housed in polypropylene cages, sterilized paper strips were used as bedding materials. Food and water were provided *ad libitum*. All the studies conducted were approved by Institutional Animal Ethical Committee according to the prescribed guidelines of CPCSEA.

Experimental Design: For this study, rats were grouped into 3, each group containing 6 rats (Table 1).

Table 1: Grouping of animals

S. No.	Experimental groups	Treatment dosage
1.	Control (n=6)	Orally treated with drinking water (1 ml/kg body weight)
2.	Test 1 (n=6)	Orally treated with *Dasamoolarishta* (1 ml/kg body weight)
3.	Test 2 (n=6)	Orally treated with 6 % ethanol (1 ml/kg body weight)

Dasamoolarishta

Dasamoolarishta is a liquid Ayurvedic medicine. For this study, it was freshly made according to the Ayurvedic Pharmacopoeia of India [23] and traditional Ayurvedic practitioners.

Preparation of *Dasamoolarishta*

Ingredients

Pure and authentic ingredients of *Dasamoolarishta* were collected from the market according to traditional Ayurvedic practioners and Indian Ayurvedic literature *Sahasrayogam* [24]. Plant specimens and the ingredients were identified and authenticated by Herbarium specialist, Department of Botany, University of Calicut.

Table 2: Composition of ingredient(s) in *Dasamoolarishta*

S.No.	Scientific name	Common name	Sanskrit name	Part used	Quantity used (g)
1.	*Gmelina arborea*	*Kumbil*	*Gambhari*	Root	18.75
2.	*Aegle marmelos*	*Koovalam*	*Bilva*	Root	18.75
3.	*Stereospermun colais*	*Padiri*	*Patala*	Root	18.75
4.	*Oroxylem indicum*	*Vellappathiri* or *Palaqapayyani*	*Syonaka*	Root	18.75
5.	*Premna integrifolia*	*Munja*	*Arani*	Root	18.75
6.	*Desmodium gangeticum*	*Orila*	*Salaparni*	Root	18.75
7.	*Pseudarthria viscida*	*Moovila*	*Chitraparni*	Root	18.75
8.	*Solanum indicum*	*Cheruvazhuthina*	*Brhati*	Root	18.75
9.	*Aerva lanata*	*Cheroola*	*Astamabayda*	Root	18.75
10.	*Tribulus terrestris*	*Njerijil*	*Goksura*	Fruit	18.75
11.	*Plumbago indica*	*Koduveli*	*Citraka*	Root	92.5
12.	*Costus speciosus*	*Pushkaramoolam*	*Pushkaram*	Root	92.5
13.	*Symplocos cochinchinensis*	*Pachotti*	*Lodhra*	Bark	75
14.	*Tinospora cordifolia*	*Chittamruthu*	*Guduchi*	Stem	75
15.	*Phyllanthus emblica*	*Nellikka*	*Dhatri*	Fruit	62.5
16.	*Tragia involucrata*	*Kodithoova*	*Duralabha*	Root	45
17.	*Acacia catechu*	*Karingali*	*Khadira*	Bark	32.5
18.	*Pterocarpus marsupium*	*Venga*	*Bandhukavriksha*	Bark	32.5
19.	*Terminalia chebula*	*Kadukka*	*Haritaki*	Seed	32.5
20.	*Saussurea lappa*	*Velutha kottam*	*Pushkara*	Root	7.5
21.	*Adenanthere pavonina*	*Manjatti*	*Manjipoovu*	Root	7.5
22.	*Erythroxylum monogynum*	*Devadarum*	*Devadaru*	Bark	7.5
23.	*Embelia ribes*	*Vizhalari*	*Vidanga*	Seed	7.5
24.	*Glycyrrhiza glabra*	*Irattimadhuram*	*Madhuka*	Root	7.5
25.	*Clerodendrum serratum*	*Cheruthekku*	*Bharngi*	Fruit	7.5
26.	*Limonia acidissima*	*Vilankaay*	*Feronia elephentum*	Fruit	7.5
27.	*Terminalia bellirica*	*Thannikka*	*Bibhitaka*	Fruit	7.5
28.	*Boerhavia diffusa*	*Thavizhama*	*Punarnava*	Root	7.5
29.	*Piper chaba*	*Kattumulaku*	*Cavya*	Stem	7.5
30.	*Nordostachys jatamansi*	*Jadamanji*	*Jatamamsi*	Flower	7.5
31.	*Syzygium cumini*	*Njaval*	*Jambu*	Flower	7.5
32.	*Hemidesmus indicus*	*Nannari* (*Naruneendi*)	*Sariva*	Root	7.5

33.	*Nigella sativa*	*Karinjeerakam*	*Krishnajeerakam*	Seed	7.5
34.	*Operculina turpethum*	*Trikalpakonna*	*Trivrut*	Root & skin	7.5
35.	*Piper cubeba*	*Arenukam* (*Valmulaku*)	*Kankolaka*	Seed	7.5
36.	*Alpinia galaga*	*Chittaratha*	*Rasna*	Seed	7.5
37.	*Piper longum*	*Thippeli*	*Pippali*	Fruit	7.5
38.	*Spaeranthus indicus*	*Adakkamaniyan*	*Hapushpa*	Seed	7.5
39.	*Curcuma zedolica*	*Kachooram*	*Sugandhamoolam*	Tuber	7.5
40.	*Curcuma longa*	*Manjal*	*Haridra*	Rhizome	7.5
41.	*Anethum graveolens*	*ShathaKuppa*	*Shathapushpa*	Seed	7.5
42.	*Caesalpinia sappan*	*Pathimugam*	*Patranga*	Bark	7.5
43.	*Mesua ferrea*	*Nagapoovu*	*Nagakesara*	Flower	7.5
44.	*Cyperus rotundus*	*Muthanga*	*Musta*	Tuber	7.5
45.	*Holarrhena antidysenterica*	*Kutakappalayari*	*Indrayava*	Seed	7.5
46.	*Pistacia integerrima*	*Karkkatakasrrhgi*	*Kuleerashrungi* (*Chakrangi*)	Seed	7.5
47.	*Cuminum cyminum*	*Jeerakam*	*Jeera*	Seed	7.5
48.	*Microstylis musifera*	*Idavakam*	*Rushabha*	Root	7.5
49.	*Polygonatum cirrhifolimum*	*Medha*	*Medha*	Root	7.5
50.	*Fritillaria roylei*	*Kakoli*	*Kakolie*	Root	7.5
51.	*Lilium polyphyllum*	*Kshirakakoli*	*Kakoli*	Root	7.5
52.	*Sida acuta*	*Kurumthotti*	*Bala*	Root	7.5
53.	*Ipomea panikulata*	*Palmutukku*	*Ksheeravidari*	Root	7.5
54.	*Vitis vinifera*	*Unakkamundiri*	*Draksha*	Fruit	225
55.	Honey	*Honey* or *Then*	*Madhu*		125
56.	*Woodfordia fructicosa*	*Thadiripoovu*	*Dhathaki*	Flower	125
57.	*Illicium verum*	*Thakkolam*	*Kakkolaka*	Seed	7.5
58.	*Plectranthus vettiveroides*	*Iruveli*	*Hroeberam*	Root	7.5
59.	*Santalum album*	*Chandanam*	*Chandana*	wood	7.5
60.	*Myristica fragrans*	*Jadikka*	*Jadiphala*	Fruit	7.5
61.	*Syzygium aromaticum*	*Grampoo*	*Lavanga*	Flower	7.5
62.	*Cinnamomum verum*	*Ilavangam*	*Twak*	Stem bark	7.5
63.	*Ellateria cardamom*	*Elakka*	*Elam*	Seed	7.5
64.	*Cinnamomum tamala*	*Pachila*	*Patra*	Leaf	7.5
65.	*Mesua ferrea*	*Nagapoovu*	*Nagakesara*	Flower	7.5
66.	*Piper longum*	*Thippali*	*Pippali*	Fruit	7.5
67.	*Jaggery*	*Vellum*	*Guda*		1500

Arishta formulation procedure

Dasamoolarishta was prepared from our department as per the Ayurvedic Pharmacopoeia of India[23] and traditional Indian Ayurvedic literature *Sahasrayogam*[24]. *Dasamoolarishta* is a fermented polyherbal preparation made with the ingredients in the formulation shown in table 2. The earthenware or pot was wiped with a clean dry cloth and then smeared with cow's ghee on the inner surface. "It prevents oozing out of the contents when left for fermentation"[11]. Raw materials were collected, cleaned, washed, dried and used for the preparation. The ingredients No: 1 - 53 (from the list) were boiled in the specified amount of potable water to get an extract or decoction, which was reduced upto one fourth of its original volume. The fruit of *Vitis vinifera* (No: 54 - *Unakkamundiri*) was separately heated in water until the quantity gets reduced to half of its initial volume. These two decoctions were filtered through a muslin cloth and collected in a clean earthenware container with ingredient No: 55 (from the list) for further processing. Then a pulverized mixture of about 10 *Podimarunnu* or *Praksepa* d*ravyas* (No: 57-66 from the list, Fig. 2 a) was added with *jaggery* (No: 67) and *Woodfordia (*No: 56, *Dhataki or Thadaripoovu,* Fig. 2 b). The earthenware was covered by a clean white cloth smeared with clay or *Puttumannu* all around the lid

(Fig. 2 c). This was allowed for a fermentation period of 30 days. The set up was covered with a wet cotton or cloth to maintain a constant moisture and temperature.

According to Ayurvedic Pharmacopoeia of India, *Dasamoolarishta* is a fermented liquid preparation made with the above ingredients and contains not more than 10 percent and not less than 5 percent of alcohol, that is self generated in the preparation over a period of time. So, the amount of alcohol content was a necessary factor, which helps to check the quality of the prepared *Dasamoolarishta.* In this study, to estimate the ethanol content of the above prepared *Dasamoolarishta* (Fig. 2 d), the sample was estimated by Semichon and Flanzy Dichromate method[25].

Ethanol estimation

The estimation procedure followed by Semichon and Flanzy Dichromate volumetric method.

Materials

1. *Dasamoolarishta* sample.
2. Standard potassium dichromate solution.
3. Standard Mohr's salt solution.
4. Potassium ferricyanide (1 %).
5. Concentrated sulphuric acid.
6. Burette.
7. Distillation apparatus.

Procedure

Dasamoolarishta sample of about 10 ml was pippetted out into a conical flask and evaporated. Ethanol was collected into an ice cold narrow flask containing powerful oxidising agent potassium dichromate. After the complete oxidation process, a brown coloured solution was obtained. Standard Mohr's salt solution was added to the brown coloured solution from a burette. The colour changes to green and finally to blue when the endpoint is reached. However, before the final colour change, add 1 % potassium ferricyanide as an outside indicator. As long as the dichromate is in excess in the solution, a drop placed on a spot plate next to a drop of ferricyanide solution forms a yellow aureole, but when the Mohr's salt is in excess, blue rays penetrate into the drop. A single drop of the standard solution produces the final colour change.

Calculation

The percentage of ethanol (P) present in the given sample was obtained by the following formula,

$$P = 20 - M / 2$$

Where 'M' is the number of milliliters of standard Mohr's salt solution required for the titration.

Dosage and administration

Normal consumption dose of *Arishta* for human is 15 - 30 ml, average of this dose was used to calculate the dosage for rats which was approximately 2.3 ml /Kg body weight[7]. For this study, a dosage of 1 ml/Kg body weight of rat was fed orally through a neonatal feeding tube.

Ethanol

Lab grade ethanol was prepared and all the chemicals used for this study were of analytical grade. Ethanol was diluted with distilled water to get the required concentration (6 %).

Neonatal feeding tube

Neonatal feeding tubes (Fig. 3) were used to force feed *Dasamoolarishta,* 6 % ethanol and drinking water for test as well as control rats respectively to induce stressful condition experienced by all the experimental animals.

At the end of the treatment (30 days), the animals were deprived of food overnight. Body weight and fasting blood glucose level were checked during early mornings. Then the animals from each group were sacrificed by decapitation. Liver was dissected out, washed in ice - cold saline, patted dry and weighed to do biochemical test for lipid peroxidation status.

Lipid peroxidation level

Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation in the cells. Malondialdehyde level is commonly known as a marker of oxidative stress. Lipid peroxidation is measured by the level of MDA, which reflects the impact of oxidative stress in cells and tissues[12]. In this study, the change in MDA level of liver tissue on *Dasamoolarishta* treated group was compared with 6 % lab grade ethanol treated groups.

Estimation of malondialdehyde

A portion of liver was weighed and homogenized with 0.1 M tris HCl to check the level of lipid peroxidation malondialdehyde. Lipid peroxidation product MDA formation was assayed by thiobarbituric reactive substance formation method [13].

Principle

Since malondialdehyde is a dehydration product of peroxidised lipids, the development of pink colour with the absorption characteristics (absorption maximum at 535 nm) as TBA-MDA chromophore has been taken as an index of lipid peroxidation.

Procedure

The tissue homogenate was prepared in 0.1 M tris HCl buffer (pH=7.5), 1 ml of homogenate was combined with 2 ml of the TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at

1000 *rpm* for 15 minutes. The absorbance of the sample was read at 535 nm against a blank that contained no tissue homogenate. The extinction coefficient of malondialdehyde is 1.56 X 10^5 m^{-1} cm^{-1} and the results were expressed as nanomoles of MDA per mg protein. Protein content of liver tissues were estimated by the method of Lowry [14].

METHODS

For this experiment, 18 healthy adult male *Wistar albino* rats weighing between 160-220 g were selected and categorized into 2 groups. Before the experiment, it is ensured that the rats were healthy by checking their body weight and blood glucose level on fasting conditions (12 hours), using a glucometer. Afterwards, from the fifth day onwards drug treatment was started for the *Dasamoolarishta* treated group, 6 % ethanol treated group (the same percentage of alcohol in *Dasamoolarishta*) and the non treated control group. Body weight of the experimental rats was checked every day and the blood glucose level was checked in a five day interval. On the continuous 25 day treatment (1 ml/kg body weight once in a day), there was no change in blood glucose level in both the control and test groups (test 1 and test 2). *Dasamoolarishta* treatment was stopped on the last day of the experiment (30th day), glucose level was checked and the rats were sacrificed after deep anaesthetization. Then the liver tissue was dissected for biochemical estimation of lipid peroxidation status by JASCO V-630 Spectrophotometer (serial No. C 395561148), Japan.

Statistical Analysis

The values are expressed as standard deviation and standard error of mean (n=6) for each group. The significant difference between groups was calculated using one-way ANOVA by Statistical Packages for Social Sciences (SPSS) version 16.

RESULTS AND DISCUSSION

Herbs are rich sources of natural antioxidants. Antioxidants terminated the chain reactions of free radicals and are thereby removing the free radical intermediates. It also inhibits the oxidation reactions. Oxidative stress and antioxidants have been weighed side by side in many diseased states. Many herbal medicines possess antioxidant properties, which play an important role in therapeutic and health management. Natural products from plant origin are said to be the best for various health related ailments because of the Phytomedicines, which have synergetic actions against pathological conditions.

According to the traditional system of Indian medicine, *Ayurveda* suggests that a combination of substances is better for therapeutics. *Churna*, *Asava, Arishta, Rasa, Parpati*, *Lepa* etc., are valuable and commonly practiced therapeutic agents in *Ayurveda*. *Dasamoolarishta*, is a commonly used *Arishta* having the ability to act on a wide range of health problems. It is also a potent stimulator of appetite, containing self generated alcohol and having a sweet taste. The fact is that this self generated alcohol does not affect adversely on health, but direct alcohol consumption like wine, beer etc., leads to its acute and chronic intoxication.

The present study compared the self generated alcohol in *Dasamoolarishta* treated and lab grade ethanol treated group rats on its malondialdehyde levels. For this comparative study, the alcohol content was first examined to ensure the purity of *Dasamoolarishta*. From the present study, the level of alcohol obtained was 5.8 - 6.0 %. The level of self generated alcohol is by the process of fermentation. It indicates that, this self generated alcohol is one of the evaluating component to determine the quality and utility of polyherbal formulations [15].

The current study carried out the comparison of the body weight, fasting blood glucose level and the level of lipid peroxidation product malondialdehyde of self generated alcohol in *Dasamoolarishta* (test 1) and 6 % ethanol (test 2) treated healthy rats. The results mentioned in the following tables and figures were compared in each of the groups.

Effect on body weight

Table 3: Comparisons of body weight of control, test 1 and test 2 groups

S.No	Groups	Days	Mean± (SD)
1.	Control (n=6)	01	203 ± 07.00
		05	203 ± 07.00
		10	214 ± 16.54
		15	229 ± 22.54
		20	241 ± 24.48
		25	249 ± 19.21
		30	251 ± 20.77
2.	Test 1 (n=6)	01	203 ± 09.68
		05	203 ± 09.68
		10	195 ± 13.18
		15	200 ± 17.52

		20	202 ± 16.02
		25	207 ± 15.26
		30	210 ± 08.67
3.	Test 2 (n=6)	01	151 ± 23.55
		05	151 ± 23.55
		10	163 ± 19.82
		15	172 ± 14.62
		20	173 ± 11.70
		25	182 ± 12.30
		30	191 ± 14.89

(SD- Standard deviation)

Table 4: Table showing multiple comparison of body weight in different groups of animals using Post hoc analysis

Days	Group	Groups	Std Error	Significance
1-5	Control	Test 1 (Arishta)	8.58616	0.045*
		Test 2 (Ethanol)	8.58616	0.000**
	Test 1	Control	8.58616	0.045*
		Test 2	8.58616	0.000**
	Test 2	Control	8.58616	0.000**
		Test 1	8.58616	0.000**
5-10	Control	Test 1	10.07196	0.000**
		Test 2	10.07196	0.000**
	Test 1	Control	10.07196	0.000**
		Test 2	10.07196	0.000**
	Test 2	Control	10.07196	0.000**
		Test 1	10.07196	0.000**
10-15	Control	Test 1	10.81075	0.000**
		Test 2	10.81075	0.000**
	Test 1	Control	10.81075	0.000**
		Test 2	10.81075	0.000**
	Test 2	Control	10.81075	0.000**
		Test 1	10.81075	0.000**
15- 20	Control	Test 1	10.62309	0.005**
		Test 2	10.62309	0.000**
	Test 1	Control	10.62309	0.005**
		Test 2	10.62309	0.000**
	Test 2	Control	10.62309	0.000**
		Test 1	10.62309	0.000**
20 -25	Control	Test 1	10.98433	0.000**
		Test 2	10.98433	0.000**
	Test 1	Control	10.98433	0.000**
		Test 1	10.98433	0.000**
	Test 2	Control	10.98433	0.000**
		Test 1	10.98433	0.000**
25- 30	Control	Test 1	10.16421	0.041*
		Test 2	10.16421	0.000**
	Test 1	Control	10.16421	0.041*
		Test 2	10.16421	0.000**
	Test 2	Control	10.16421	0.000**
		Test 1	10.16421	0.000**

** Significant at the 0.01 level (1 %)

* Significant at the 0.05 level (5 %)

Table 3 shows the mean and standard deviation of the body weight (gm per day) of the control (drinking water treated), test 1 (*Dasamoolarishta* treated) and test 2 (ethanol treated) rats. Before the treatment with *Dasamoolarishta* treated (test 1) group, body weight was found to be the same as the untreated control groups. At the end of the experiment, (on the 30th day) there is a slight variation of body weight in both the *Dasamoolarishta* treated and ethanol treated test groups. From table 4, in a multiple comparison by post hoc analysis of control group with the *Dasamoolarishta* treated group, there was a sudden change in the control group body weight, but does not affect the body weight of *Dasamoolarishta* treated group. When control group was compared with ethanol treated group, there was a similar effect of body weight that progressively increased. In the control group, the body weight increased gradually till the end of the experiment. Animals which consumed higher amount of alcohol showed a lower body and liver weight due to fat mass reduction [16]. From the analysis, a continuous 25 day treatment (5th day onwards) in *Dasamoolarishta* treated group when compared with the control group was found statistically significant at 5 % ($p< 0.05$) level and a comparison of *Dasamoolarishta* treated group with ethanol treated group was statistically significant at 1 % ($p<0.01$) level.

Effect on Blood Glucose Level (BGL)

One of the studies showed that there was an increase in the blood glucose level in alcohol treated healthy and stress induced alcohol treated rats [17]. A comparison of BGL of control, test 1 and test 2 groups are represented in Fig. 4. From this study, before the treatment (1st day) blood glucose level was found to be normal in all the tested animals. After a consecutive drug treatment for 25 days, there was a significant reduction in the blood glucose level in all the groups. On the 30th day of the experiment, the mean and standard error (Mean ± SE) of fasting blood glucose level of control, test 1 and test 2 are 117 ± 6.08, 115 ± 6.00 and 119 ± 14.40 respectively. From the analysis, there was no statistically significant difference between each of the group. Hence, the present study does not show any deviation of normal blood glucose level even in the test group (test 1 and 2) rats.

Effect on Lipid peroxidation

Liver damage like fatty liver, cirrhosis and hepatitis may be because of alcohol ingestion[18]. Direct consumption of alcohol is also associated with elevated level of lipid peroxidation. Lipid peroxidation of unsaturated fatty acids is frequently used as an indicator of oxidative stress and subsequent oxidative damage. Poly unsaturated lipid peroxidation causes molecular damage through its free radical intermediates[19]. Levels of MDA were found to be increased in toxicity induced rats as compared to normal rats[20]. An increase in free radicals causes overproduction of MDA. A few of the study proved that "plant derived products or phyto constituents are found to be effective as free radical scavengers and inhibitors of lipid peroxidation"[21-22].

Fig. 5, depicts the level of lipid peroxidation product malondialdehyde in control, test 1 and test 2 groups. Hepatic MDA level in different groups of animals (mean ± SE (SE-Standard Error)) such as control, test 1 and test 2 are 0.6572 ± 0.09, 0.6844 ± 0.07 and 0.9879 ± 0.10 respectively. The ethanol treated rats (test 2) showed significantly elevated levels of MDA when compared with non treated control and *Dasamoolarishta* treated (test 1) group. Treatment with herbal formulations like *Dasamoolarishta* reduced the level of lipid peroxidation products like malondialdehyde.

CONCLUSION

Poly herbal formulations is also important in the management of health, because of having a potence to reduce free radicals from oxidative damages under normal healthy conditions. Present study reveals that *Dasamoolarishta* treated group showed a reduced malondialdehyde level than the ethanol treated groups. This comparative study suggests that the use of *Ayurvedic* preparation *Dasamoolarishta,* is not only acting isolated, but also giving a synergetic action against naturally producing free radicals. Further molecular studies are needed at the level of antioxidant biomarkers in an induced stressful condition**.**

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Cite this article as:
Thadathil Pandarekandy Seena, Padinjareveedu Subrahmanyan Surya, Girijavallabhan Kannamparathazhathethil Sneha, Abdulrahiman Rahmath, Edakkot Sreekumaran. Comparative Study of Oxidative Stress Marker Malondialdehyde on Dasamoolarishta and Ethanol Treated Healthy Wistar Albino Rats. International Journal of Ayurveda and Pharma Research. 2015;3(7):22-31.

Source of support: Nil, Conflict of interest: None Declared

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Figure 2 (2a – 2d): Ingredients and preparation of *Dasamoolarishta by* traditional methods

Figure 1: Experimental animal *Wistar albino* male rat

Figure 2a: *Prakshepa dravya* or *podimarunnu* 57 – 66 from the list

Figure 2b: *Woodfordia fruticosa* flowers

Figure 2c: Fermentation in earthenware pot

Figure 2d: Final product in bottle

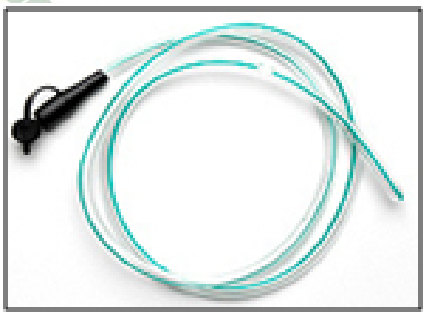


Figure 3: Neonatal feeding tube for oral drug administration

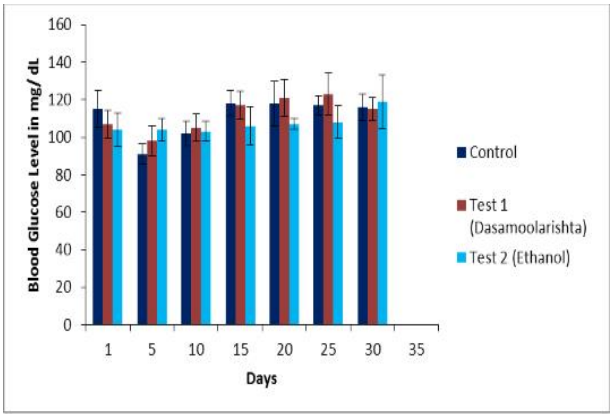
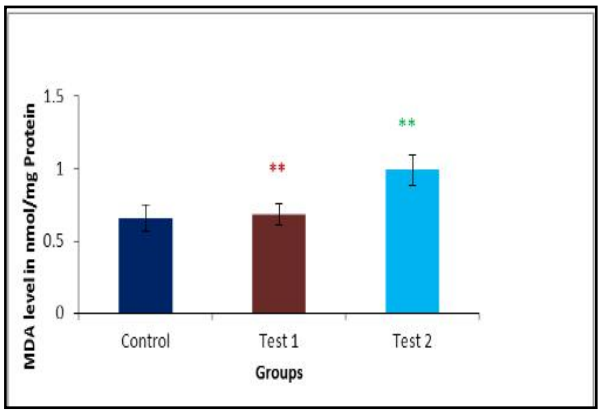


Figure 4: Showing the comparison of blood glucose level in control (non treated), test 1 (*Dasamoolarishta* treated) and test 2 (lab grade 6 % ethanol treated) groups



** Comparison of hepatic MDA level between control and test 1 groups.*

** Comparison of hepatic MDA level between test 1 and test 2 groups.*

Figure 5: Showing the comparison of Lipid peroxidation product malondialdehyde level in control (non treated), test 1 (*Dasamoolarishta* treated) and test 2 (lab grade 6 % ethanol treated) groups