COMPARATIVE ANTILIPIDEMIC EFFECTS OF NATIVE AND GEMMO-TREATED *WITHANIA SOMNIFERA* (ASGHAND*)* EXTRACTS

Fouzia Khursheed[1], Khalil-ur-Rehman[1], Muhammad Shoaib Akhtar[2], M. Zahoor-ul-Hassan Dogar[3], Bilal Khalil[1]

1. Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
2. Pharmacology Department, Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan
3. Department of Biochemistry, Sargodha Medical College, University of Sargodha, Sargodha-Pakistan

ABSTRACT

Antilipidemic activity was measured by estimating the levels of lipidemic markers such as total, HDL & LDL cholesterol, and triglycerides, before and after treatment with Gemmo and extracts from native plants. It was observed that antilipidemic activity were more efficient in Gemmo treated groups as compared to natively treated animals. Moreover, Gemmo extracts exerted significant antilipidemic effect in isoproterenol-induced myocardial infarction by maintaining the level of total, HDL & LDL cholesterols and triglycerides. Antilipidemic activity of *Withania somnifera* might be due to alkaloids and special compounds called Withanolides. It is conceivable that that these plant constituents are probably more active in form of Gemmo-extracts than in the native form.

Keywords**:** *Withania somnifera* (Asgand); Gemmo-treatment; Total HDL-cholesterol and LDL-cholesterol; Triglycerides; Withanolide alkaloids; Antilipidemic activity

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Address for Correspondence**:** Prof Dr Muhammad Shoaib Akhtar, Department of Pharmacology, Faculty of Pharmacy, University of Sargodha, Sargodha-48010, Pakistan. Email Address: drmsakhtar@gmail.com

INTRODUCTION

Plant-based pharmaceuticals have been employed since centuries in the management of various diseases of man and animals (Temple *et al*., 1996). The primary benefit of using plant derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits at more affordable rates (Aarts, 1998). Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Cragg *et al*., 1999). WHO has pointed out that 70-80% of world's population use medicinal plants (Stephen, 1999) and so far around 35,000 plants species have been used for medicinal purposes (Narayan *et al*., 2003).

Recently focus on plant research has intensified all over the world. Large amount of evidence have been collected to show immense potential of medicinal plants used in various traditional systems (Dahanukar *et al*., 2000). A large number of medicinal plants have already been tested and found to posses active principles useful for treating various diseases (Haider *et al*., 2001). Moreover, several plants produce secondary metabolites, which possess biological and pharmaceuticals activities and have played important role in development of new drugs (Verpoorte, 2000).

Gemmo-therapy is a lesser known but modern and exciting research division of phyto-therapy which uses stem cells of buds and young shoots of trees and shrubs, gathered in the spring time when they are at a key stage of their natural growth cycle. Young shoots and buds of medicinal plants are freshly prepared in a process using water, glycerin and alcohol. Since these extracts are from fresh and growing tissues, Gemmo-therapeutic remedies are unique in their intense combination of vitamins, minerals and other powerful properties of whole plants, including flowers, leaves, fruits, sapwood and roots.

Cardiovascular diseases are usually caused by a blockage in the circulatory system that prevents blood from flowing to the heart or the brain. The most common cause is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain. The blood vessels become narrower and less flexible, also known as atherosclerosis (Braunwald *et al.,* 1997). Globally, myocardial infarction is a major public health concern and leading cause of mortality (Tilak-Jain and Devasagayam, 2006). In recent years, an increasing number of young people are succumbing to myocardial infarction due to unusual risk factors characterized by high triglycerides, low high density lipoprotein cholesterol (HDL-C), glucose intolerance, insulin resistance abdominal obesity and increased lipoprotein levels (Farvin *et al*., 2006; Packard *et al*., 2005). The major abnormalities noticed following myocardial infarction are lipidemia, peroxidation and loss of plasma membrane integrity (Farvin *et al*., 2004). There is well-established relation-ship between coronary artery diseases, serum lipid and lipoproteins levels (Haust and More, 1972).

Withania somnifera (Family: Solanaceae) is cultivated in Pakistan and India. The main constituents of this plant are alkaloids and steroidal lactones. It has been reported to possess anti-carcinogenic and antioxidant properties and is being used as drug (Ichikawa, *et al*. 2006). In Ayurvedic medicine, it is used as a home remedy for several diseases and is also mentioned as a herbal tonic and health food (Sharma *et al*., 1985). It is known to contain alkaloids and a special group of compounds called Withanolides, which are considered to be its active principles (Tripathi *et al.,* 1996). Recently, it has been shown to inhibit lipid peroxidation in stress induced animals (Dhuley, 1998). In this present study, we have attempted to assess comparative preventive and curative antilipidemic effects of Gemmo-therapeutically-treated *Withania somnifera* in the experimental model of *salbutamol*-induced myo-necrosis and have determined their effects on total, HDL-C, LDL- Cholesterol and triglycerides.

MATERIAL AND METHODS

Sample Collection & Extract Preparation

Samples of *Withania somnifera* were collected from botanical gardens of University of Agriculture, Faisalabad and were identified by the Plant taxonomist in Department of Botany, University of Agriculture Faisalabad, Pakistan.

Native Extract

Withania somnifera leaves were washed with distilled water. After drying in a shed, about 500 g of clean leaves were added to 250 ml of H_2O, shacked and boiled for half an hour, taken into the beaker

and was left for one day. Next day the solution was filtered and filtrate was used for the dose preparation of rabbits.

Gemmo Extract

Fresh growing leaves were washed with distilled water and weighed. The samples were set aside so that the water will become evaporated and the exact weight of plant material was determined. The fresh plant material blended in a mixture of alcohol and glycerin having a ratio of 2:1, respectively. The mixture was left to stand for one month in a cool, shaded environment, and shaken from time to time to help the maceration process. It was then filtered under constant pressure. After standing for a further forty eight hours, it was filtered ones again. The resulting liquid was known as the stock, it consists of glycerin and alcohol in a ratio of 2:1 respectively. The stock solution was then evaporated in rotary so that all the alcohol was removed. The solution was kept in an incubator at a temperature of 65° C, and thus the remaining alcohol was be evaporated. This stock solution or extract (Gemmo-therapeutically treated *Withania somnifera*) was used within five years from the date on which the plant material was picked (Churchill, 2002).

Phyto-chemical Screening

Qualitative and quantitative analysis was done for identification of the major phyto-constituents of *Withania somnifera* and was carried out as described by Brain and Turner (1975) and Siddique and Ali (1997).

Animal Study

Salbutamol plant extracts (Gemmo and native), syringes, cotton, centrifuge tubes, Eppendorf tubes, kits of total LDL,HDL cholesterol and triglyceride were required.

Eighteen rabbits weighing about 1.25 kg were used in the study. Animals were kept in cages under hygienic and standard environmental conditions. They were fed a regular rabbit chow and water *ad libitum*. Animals were weekly weighed and were randomly allocated eight groups comprising three animals each. Table I shows the treatment protocol followed in the study.

Biochemical & Pathological Tests

Blood samples were collected from jugular vein of rabbits (Behne, 1981) before starting experiment, during and after 30 days of experiment. The blood samples were centrifuged and serum was separated and stored in deep freezer for further biochemical measurements. Serum was used for the assay of biochemical parameters including total cholesterol, low density lipoproteins (LDL) cholesterol, high density lipoproteins (HDL) cholesterol and triglycerides (TAGS) using kits.

Table – I: Treatment protocols followed in the experiments

Groups	Day 1-20	Day 20th	Day 21st	5 days After ischemia
1) Normal	normal diet	normal diet	normal diet	--
2) Ischemia	normal diet	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	--
3) *WS* Gemmo baseline	Gemmo-extract (50 mg/ kg)	--	--	--
4) *WS* native baseline	Native-extract (50 mg/ kg)	--	--	--
5) *WS* Gemmo-preventive	Gemmo-extract (50 mg/ kg)	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	--
6) *WS* Native-preventive	Native-extract (50 mg/ kg)	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	--
7) *WS* Gemmo-curative	normal diet	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	Gemmo-extract (50 mg/ kg)
8) *WS* Native-curative	normal diet	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	Native-extract (50 mg/ kg)
9) *WS* Gemmo-curative	normal diet	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	Gemmo-extract (50 mg/ kg)
10) *WS* Native-curative	normal diet	Salbutamol (50mg/ kg)	Salbutamol (50mg/ kg)	Native-extract (50 mg/ kg)

In the end, gross pathology of experimental animals was done under supervision of a Veterinarian. Apparent changes in weight and structure of heart, kidneys, liver, stomach and lungs were noted The data were analyzed statistically by using analysis of variance (ANOVA) and P values <0.05 has been considered as significant (Steel *et al.,* 1997).

RESULTS

Phytochemical Constituents

Table II shows results of qualitative phytochemical analysis of native and Gemmo modified extract of the plant, which shows that common constituents present in large amounts were flavonoids, glycosides and tannic acid, etc.

Table – II: Quantative analysis of phyto-constituents present in *Withania somnifera*

Serial No.	Phytoconstituents	Qualitative Analysis	
		Gemmo extract	Native Extract
1	Alkaloids	+	++
2	Flavonoids	+++	++
3	Glycosides	++	+
4	Tannic Acid	+++	+
5	Saponins	+	+
6	Steroids & Triterpenoids	+	+

(–) Not detected; (+) present in low concentration; (++) present in moderate concentration; (+++) present in high concentration.

Cardio-protective effects

Oral administration of s*salbutamol* (50mg/ kg) significantly increased ($p<0.05$) heart rate; in Gemmo-curative (115 beats/ 30 sec) and native-curative (112 beats/ 30 sec) as compared to normal (95 beats /30 sec). Heart beat of rabbits was checked regularly with an interval of 24 hours before and after administration of *salbutamol*. Post-treatment of ischemic rabbits with Gemmo and native extracts of *Withania somnifera* (50 mg/ kg) for five days significantly reduced ($p<0.05$) heart beat both in Gemmo-curative (90 beats /30 sec) and Native-curative groups (98 beats /30 sec) as compared to ischemic group (112 beats /30 sec). Gemmo-extract showed more significant results (90 beats /30 sec) than native-extract (97 beats /30 sec).

Table – III: effects of *Withania somnifera* on different blood lipid levels (mg/dl)

Days	Normal	Not Treated	Gemmo-treated curative	Native-treated	Gemmo-preventive	Native-preventive
1	94	89	85	95	93	
2	93	96	92	101	99	97
3	94	100	105	103	97	103
4	95	118	115	112	101	110
5	95	120	111	112	99	116
6	94	121	105	118	95	112
7	95	119	99	105	96	107
8	93	115	93	99	94	101
9	95	112	90	97	94	98
						96

Similarly in preventive therapy, pre-treatment of ischemic rabbits with Gemmo and native extracts of *Withania somnifera* (50 mg/ kg) for 20 days significantly restored ($p<0.05$) the heart beat to normal, both in Gemmo-preventive (94 beats /30 sec) and Native-preventive groups (95 beats /30 sec) as compared to ischemic group (112 beats /30 sec). Both the Gemmo and native-extract showed similar cardio-protective effects. The *Table III* shows the Tachycardia values (per 30 sec) of different groups.

Rabbits were pre-treated with native and Gemmo-extract of *Withania somnifera* for 20 days and then treated with s*albutamol* (50mg/kg) once a day for two days to induce myocardial injury.
The effects native and Gemmo modified extracts of *Withania somnifera* on various fractious of blood lipids are shown in Table III. Similarly in native-preventive group, pre-treatment with the native extract of *Withania somnifera* significantly restored the serum enzyme levels to normal; cholesterol (237.00±10.09) HDL cholesterol (76.32±17.58), LDL Cholesterol (128.67±14.19) and triglycerides (210.0±9.58) as compared to ischemia group having serum enzyme levels of the cholesterol (357.00±17.10) HDL cholesterol (32.33±1.45), LDL cholesterol (134.67±60.88) and triglycerides (425.00±20.5), respectively.

Table – IV: Preventive effect of *Withania somnifera* on different enzyme levels (U/L)

Lipids	Phase	Normal Control	Non treated Ischemic	Gemmo prevented	Native prevented	Gemmo-treated prevented	Native-treated prevented
Total Cholesterol	Start						
	End	202.67±7.80	357.00±17.10	198 ± 5.99	190 ±12.051	214.00±6.51	237.00±10.09
HDL Cholesterol	Start						
	End	64.33 ± 15	32.33 ± 1.45	62.15 ± 3.12	63.33 ± 13.45	67.33 ± 12.33	76.32 ± 17.58
LDL Cholesterol	Start						
	End	114.33±7.22	134.67±20.88	110.20±6.37	112.78±8.03	120.33±1.86	128.67±14.19
Triglycerides	Start						
	End	167.33 ± 5.49	425.00±20..50	164 ± 3.59	169 ± 6.25	171.33±6.51	210.00±9.58

Both the Gemmo- and native extracts of *Withania somnifera* have showed antilipidemic activity and significantly restored lipid levels in Gemmo-preventive and native-preventive groups to normal levels.

Curative effects

Table – V: Curative effect of *Withania somnifera* on different lipid levels (mg/dl).

		Normal Control	Non Treated Ischemic	Gemmo treated - curative	Native treated
CHOLESTEROL	Day-1	225.67±7.80	346.33±20.85	324.671±8.33	341.67±6.77
	Day-2	22.00±7.51	357.00±17.10	320.67±28.22	323.33±6.84
	Day-3	222.00±5.78	375.00±8.95	298.67±11.84	290.67±15.34
	Day-4	223.67±6.94	398.67±3.93	272.33±20.48	267.67±18.41
	Day-5	230.67±3.67	425.00±8.50	231.00±8.33	245.00±12.06
HDL	Day-1	64.33±3.38	55.33±1.45	33.67±.67.0	61 .33±5.78
	Day-2	65.33±2.67	52.33±0.67	39.33±4.63	54.67±5.88
	Day-3	62.67±0.88	47.33±1.20	47.67±2.96	57.67±1.20
	Day-4	62.33±8.8	41.67±1.20	59.33±5.81	62.67±2.03
	Day-5	62.00±1.53	34.67±20.3	82.00±58.0	70.00±4.04
LDL	Day-1	114.33±7.22	134.67±0.88	126.67±2.86	130.33±5.40
	Day-2	114.670±6.36	145.33±2.67	27.67±1.87	142.67±4.36
	Day-3	113.33±6.69	156.00±1.53	122.0±4.51	125.00±4.69
	Day-4	114.00±5.51	153.331±.20	117.67±1.45	129.33±2.03
	Day-5	113.67±6.84	150.67±1.20	113.33±4.70	123.67±1.45
TRIGLYCERIDES	Day-1	167.33±5.49	246.33±20.85	239.67±17.33	241.67±18.67
	Day-2	165.67±706	258.67±18.62	260.67±16.22	238.67±20.17
	Day-3	164.67±6.69	253.67±13.12	233.002±4.13	239.671±15.34
	Day-4	171.00±4.16	246.00±12.50	171.00±7.64	220.33±13.87
	Day-5	223.67±3.76	240.00±8.50	166.33±5.57	203.00±10.58

Ischemia was induced in rabbits by oral administration of *Salbutamol* (50mg/ kg) for two consecutive days at an interval of 24 hours. *Salbutamol* significantly increased ($p<0.05$) the serum levels of Total, LDL Cholesterol, Triglycerides and decreased the serum level of HDL Cholesterol in curative groups as compared to normal. However, post-treatment of ischemic rabbits with Gemmo and native extracts of *Withania somnifera* (50mg/ kg) significantly reduced ($p<0.05$) serum levels of Cholesterol, LDL Cholesterol, Triglycerides and Increased the serum level of HDL Cholesterol in curative groups as compared to ischemia group in a five-day trial. *Table V* shows the total, HDL, & LDL cholesterol and triglycerides levels in mg/dl of different groups. Results showed that Gemmo extract was more effective than the native extract.

Administration of salbutamol (50 mg/kg) for two consecutive days significantly ($p<0.05$) increased serum level of total cholesterol in Gemmo-curative group (324.671±8.33) and native-curative group (341.67±6.77) as compared to non-treated group (346.33± 20.85) while in normal group (225.67±7.80). Post-treatment of ischemic rabbits with Gemmo and native extracts of *Withania somnifera* (50 mg/kg) for five days significantly ($p<0.05$) reduced serum levels of total Cholesterol both in Gemmo-curative (231.00±8.33) and Native-curative groups (245.00±12.06) as compared to non treated ischemic group (410.00±8.50) and in normal control group (230.67 ± 3.67) mg/ul, respectively. Gemmo-extract showed more significant value (231.00±8.33) than native-extract (245.00±12.06).

Subsequent to the administration of Salbutamol (50 mg/kg) serum level of HDL-C decreased significantly ($p<0.05$) in Gemmo-treated group (44.67±.67.0) and native-curative group (61.33±5.78) as compared to non treated group (55.33 ± 1.45) and in normal group (64.33±3.38). However, post-treatment of ischemic rabbits with *Withania somnifera* for five days ($p<0.05$) did not decrease rather increased serum levels of HDL cholesterol both in Gemmo-curative (82.00±58.0) and Native-curative groups (70.00±4.04) as compared to non treated ischemic group (34.67±20.3) and normal control group (62.00 ± 1.53) mg/ul while Gemmo-extract showed more significant results (82.00±58.0) than native-extract (70.00±4.04).

Oral administration of Salbutamo*l* (50mg/kg) for two consecutive days significantly ($p<0.05$) increased serum levels of LDL-C in Gemmo-curative (126.67±2.86) and native-curative groups (130.33±5.40) as compared to untreated ischemic (134.67±0.88) and in normal group (114.33 ± 7.22). However, post-treatment of ischemic rabbits with *Withania somnifera* for five days significantly reduced ($p<0.05$) serum levels of LDL-C both in Gemmo-curative (113.33±4.70) and Native-curative groups (123.67±1.45) as compared to non treated ischemic group (150.67±1.20) and normal control group (113.67 ±6.84) mg/ml, respectively. Gemmo-extract showed more significant result (113.33±4.70) than native-extract (123.67±1.45 mg /dl) at the end.

Administration of Salbutamol (50mg/kg) for two consecutive days significantly increased serum level of triglycerides in Gemmo-curative group were 239.67±17.33 and 241.67±18.67 in native-curative group as compared to non treated ischemic group (246.33 ± 2085) and non treated ischemic group (246.33 ± 20.85) normal group (167.33±5.49). Post-treatment of ischemic rabbits with *Withania*

somnifera for five days significantly reduced ($p<0.05$) serum levels of triglycerides both in Gemmo-curative (166.33±5.57) and Native-curative groups (203.00±10.58). The comparison showed. Gemmo extract more effective than native-extract.

Pathological Effects:

Gross pathology refers to macroscopic manifestations and immediately after sacrificing, the animals gross pathology changes were studied by a veterinary doctor, various pathological effects produced are shown in Table VII & VIII.

Table – VII: Gross pathological studies of different organs of rabbits

Organs	Normal Control	Diseased (after Salbutamol 50 mg / KG / day for 5 days)						
		Non treated	Gemmo treated	Native treated	Gemmo prevented	Native preventive	Gemmo prevented + treated	Native prevented + treated
Heart	Normal	Hard (damage)	Normal	Hard	Normal	Normal	Normal	Normal
Liver	Normal	Normal/ pale yellow	Pale yellow	Discolored	Normal but discolored	Normal but discolored	Normal	Normal
Kidney	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Stomach	Normal	Damage	Damage	Damage	Normal	Normal	Normal	Normal
Lungs	Pale red	Congested	Normal	Congested	Normal	Pale red	Normal	Pale Discolored

Table – VIII: Weights of different organs of rabbits of different groups

Organs	Normal	Diseased (after Salbutamol 50 mg / KG / day for 5 days)						
		Ischemia	Gemmo-treated	Native-curative	Gemmo-preventive	Native-preventive	Gemmo prevented + treated	Native prevented + treated
Heart	2.13	2.63	2.22	2.81	3.44	3.01	3.4	3.0
Liver	26.17	29	28	27.74	29.1	28.45	28.5	26.4
Kidney	7.9	7.85	7.35	7.27	7.14	7.30	6.82	7.2
Stomach	4.75	5.03	5.8	4.91	5.23	5.01	5.02	4.96
Lungs	6.4	5.8	6.48	6.3	6.1	6.03	6.1	6.02

DISCUSSION

Results obtained showed dose-related lowering effects of *Withania somnifera* extract on serum Total, LDL-cholesterol and triglycerides but dose-related elevation of serum HDL-C. Our plant showed maximum flavonoids and tannins contents, which strongly suggest the antioxidant and cardioprotective, potential of *Withania somnifera.* (Rastogi and Mehrotra, 1991; Ju, 2005; Lie and Chiou, 1986).

As described above a decrease in the serum levels of Total LDL-Cholesterol, Triglycerides and increase in serum level of HDL Cholesterol was observed in Salbutamol treated groups. Pre and post-treatment with native and Gemmo-therapeutically treated *Withania somnifera* (50 mg/Kg) restored the level of lipids to normal levels. The base-line contents were found to be normal, which reveals that W. *somnifera* at a dose 50mg/ kg did not induce any cardio-toxic effects. This is according to the previous report presented by Mohanty *et al.* (2004) that *W. somnifera* at 50 mg/kg dose produced maximum antilipidemic effects. The decrease in serum total, LDL-C and triglycerides and increase in the levels of HDL-C may be due to presence of high flavonoid content in Gemmo extract of *Withania somnifera* . The presence of high levels of flavonoids may suggest a wide spectrum of biological actions including hypoazotemic, hypotensive, hypo-glycemic, estrogenic, spasmolytic, cholagogic, anti-inflammatory, antilipidemic and antioxidant activities (Oladele *et al*., 1995). The hypoglycemic and hypolipidemic activities of flavonoids from different plant sources have also been reported. (Sudheesh *et al*., 2005). The presence of flavonoids in high concentrations in the extract may account for its hypoglycemic and antilipidemic effects. It was reported that flavonoids play a major role in reducing the risk of cardiovascular diseases by decreasing the blood lipids. Our results showed that flavonoids mixture significantly decreased the plasma triglycerides, total cholesterol, and free fatty acids with increasing HDL cholesterol level (Narender *et al.,* 2006).

Gemmo extract showed more antilipidemic potential than native extract in curative therapy with similar results in preventive therapy. Antilipidemic effects of Gemmo-extract is significant than native-extract and is probably due to embryonic part of the plant being particularly effective for drainage and detoxifying actions on the human body. Synthetic pharmaceutical agents, most herbs, and homeopathic remedies that are prepared from the whole plant (usually flowering) have less amount of many of the key elements (growth factors, phyto-hormones and auxins) formed during growth stage of plants. This is because the gemmae contain many active principles that start to disappear after a plant reaches a certain stage of development (Daniel and Towle, 2002).

The gross pathological examination of different organs of animals suggests cardioprotective potential of *Withania somnifera*. Gross pathological confirmation of cardio-toxic effect produced by salbutamol (50 mg/kg), in the present investigation has established the suitability of this model for studying the cardioprotective effect of *Withania somnifera* (Mohanty *et al.,* 2004). Compared to curative therapy, gross pathology findings showed relatively greater efficacy of preventive therapy. The histopathological examination showed that Gemmo-extract showed results that are more significant in

curative therapy. Hemalatha *et al* (2006) have reported that histopathological examination of liver tissues of treated hyperlipidemic rats showed lesser degenerative changes as compared with hyperlipidemic controls.

The major active constituents of *Withania somnifera* exhibit medicinal actions of certain steroidal alkaloids and lactones as a class and are called Withanolides. The root contains steroidal lactone (Withaferin A) and related Withanolides, along with various alkaloids. It is reported that Sitoindosides VII, VIII, IX and X are most probably adaptogenic active substances present in *Withania somnifera*. The exact mechanism of such myocardial adaptation is not yet known. However, it has been proposed to function through induction of certain antioxidant agents (Archana and Namasivayam, 1999).

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