

Research Article

Aqueous Bark extract of *Terminalia arjuna* protects against Cigarette Smoke-induced alterations in Serum Lipid Profile of Albino Rats

Rajni Singh* and Asha Agarwal

Department of Zoology, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra.

Abstract: The present study aimed to evaluate the protective effect of aqueous bark extract of *Terminalia arjuna* against cigarette smoke-induced changes in serum lipid profile of albino rats. The rats were divided into three groups. One control group (I) and two experimental groups (II and III), all three groups consisting of five rats each. The control group (I) was unexposed to cigarette smoke. Experimental group (II) was exposed to cigarette smoke (6 cigarettes) for one hour/day for 30 days and experimental group (III) was exposed to cigarette smoke along with oral administration of aqueous bark extract of *Terminalia arjuna* (5mg/rat/day) for 30 days. The findings showed a significant ($P<0.01$) increase in serum cholesterol, serum triglyceride, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with significant ($P<0.01$) decrease in high-density lipoprotein (HDL) level in cigarette smoke-exposed albino rats in comparison to control rats while a significant ($P<0.01$) decrease in serum cholesterol, serum triglyceride, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with significant ($P<0.01$) increase in high-density lipoprotein (HDL) after oral administration of aqueous bark extract of *Terminalia arjuna* in comparison to cigarette smoke-exposed albino rats.

Keywords: Cigarette smoke, Albino rat, Serum lipid profile, Aqueous bark extract of *Terminalia arjuna*.

1. Introduction

Cigarette smoking is one of the most extensively used potentially hazardous social habits throughout the world (Patel *et al.*, 2014). It is one of the biggest threat to current and future world health (WHO, 2008). About more than 5 million people die from smoking-related illness. Cigarette smoking is most important modifiable risk factor for atherosclerosis and increasing morbidity and mortality of chronic heart disease (CHD). Cigarette smoke has over 4000 toxic and mutagenic components such as carbon dioxide, aromatic hydrocarbons and nicotine (Carvalho *et al.*, 2006). Cigarette smoke contains a range of oxidant and free radicals that can increase lipid peroxidation. Free radicals can, directly and indirectly, induce oxidative stress in the body (Hecht, 2002). The oxidation reactions can produce free radicals, which start chain reaction and subsequently can cause a large number of cardiovascular disease.

Terminalia arjuna used as an antioxidant is a remarkable tree for its important phytochemicals belongs to the family Combretaceae (Singh *et al.*, 2004). The plant is a very rich source of natural antioxidants due to which it has been extensively

investigated for its uses in both Ayurvedic and Unani system of medicine (Prakash *et al.*, 2007). Therefore the present study was designed to investigate the aqueous bark extract of *Terminalia arjuna* on lipid profile after exposure to cigarette smoke in albino rats.

2. Material and Methods

The adult healthy rats of weight ranging from 150-200g of both the sexes were kept in polypropylene cages and maintained at standard laboratory conditions of temperature $21\pm 0.5^{\circ}\text{C}$ and relative humidity $60\pm 5\%$ with a photoperiod 12 hours/day. The experimental protocol was in accordance with institutional ethics committee. The rats were fed on commercial food pellets (Golden Feeds, New Delhi) and water *ad libitum*. The experimental animals were acclimatized for one week prior to the experiment.

2.1 Selection of cigarette

The Capston pilot (a filtered cigarette of 64mm length), ITC Limited, Kolkata was selected for the present study.

2.2 Plant material and extraction

The *Terminalia arjuna* bark was collected from botanical garden of School of Life Sciences, Khandari campus, Agra and identified by a plant taxonomist. Heat distillation process was used. The stem bark powder was boiled in water at 1:5 ratio at 100°C for 30 minutes (Sonia *et al.*, 2001). After 30 minutes the mixture was filtered and the filtrate was stored in refrigerator until use.

2.3 Experimental design

The albino rats were divided into three groups – one control (I) and two experimental groups (II and III) containing five rats each. Control group (I) unexposed to cigarette smoke. Experimental group (II) exposed to 6 cigarettes/hour in a day (1 cigarette/10 minute) for 30 days. Experimental group (III) pre-treated with oral administration of aqueous bark extract of *Terminalia arjuna* (5mg/100g.b.wt.) before 30 minutes, then exposed to cigarette smoke for one hour/day for 30 days.

2.4 Exposure to cigarette smoke

Mini exposure cabinet (60cm x 30cm x 30cm) manufactured by Precision Instruments, Varanasi is used for cigarette smoke exposure. The experimental rats were kept in an isolated smoke chamber with their cages for whole body exposure to cigarette smoke of a filtered cigarette (6 cigarettes/hour in a day) for 30 days.

2.5 Blood collection

Blood samples were collected from the ventricle of heart in sterilized plain centrifuge tubes and were centrifuged at 2500 rpm for 30 minutes. Serum was

separated for the estimation of lipid profile in control and experimental sets.

2.6 Estimation of serum lipid profile

Serum cholesterol was estimated by Roeschlau *et al.*, (1974), serum triglyceride by Schettler and Nussel (1975) method, HDL by Wybenga *et al.*, (1970) method, LDL and VLDL were calculated by Friedewald *et al.*, (1972) methods. The data were expressed as Mean \pm SEM. They were signified by using 't' test. By KpKy plot (Version 3.0).

3. Results and Discussion

Data obtained for serum lipid profile in control and experimental groups are given in (Fig. 1 and Table 1). According to the obtained results from this study, there was a significant increase in serum cholesterol, serum triglyceride, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with significant decrease in high-density lipoprotein (HDL) level in cigarette smoke-exposed albino rats in relation to control group.

Cigarette smoking is associated with an increase in the level of serum cholesterol, serum triglyceride, LDL and VLDL and decrease in HDL due to a decrease in lipoprotein lipase activity (Reaven and Tsao, 2003). Cigarette smoke is one of the factors in oxidant production and free radicals. Free radicals can increase oxidized LDL (ox-LDL) via different mechanisms such as lipid peroxidation, inhibition of protein production and the reduction of adenosine 3'-phosphate on the effect of lipid peroxidation (Soleimani and Rahmani Kahnamoie, 2014).

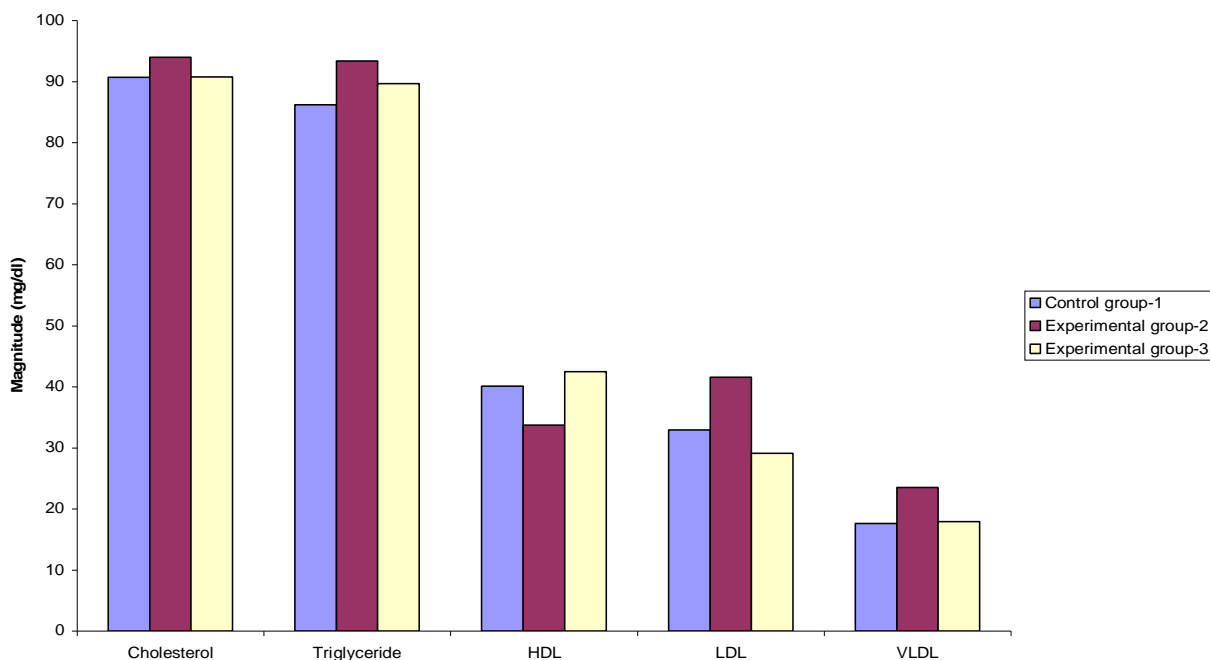


Fig. 1. Effect of cigarette smoke on lipid profile and its modulation by aqueous bark extract of *Terminalia arjuna* after 30 days exposure.

Table 1. Effect of cigarette smoke on lipid profile and its modulation by aqueous bark extract of *Terminalia arjuna* after 30 days exposure.

Groups	Treatment	30 days (Mean ± SEM)				
		Cholesterol	Triglyceride	HDL	LDL	VLDL
Control group-1 (5)	Ambient air	90.704±0.435	86.18±1.522	40.13±2.60	32.94±2.83	17.634±0.299
Experimental group-2 (5)	Cigarette smoke	93.966±0.901 ↑**	93.386±0.472 ↑**	33.72±2.57 ↓**	41.56±2.934 ↑**	23.55±0.033 ↑**
Experimental group-3 (5)	Cigarette smoke + aqueous extract	90.786±0.245 ↓* ↓••	89.696±0.790 ↑* ↓••	42.52±1.01 ↓* ↑••	29.09±1.25 ↑* ↓••	17.956±0.166 ↑* ↓••

(5) = No. of albino rats
 Difference from control
 * - Non-significant,
 ** - Significant
 Difference from cigarette smoke exposure
 • - Non-significant
 •• - Significant

Nicotine increases the circulatory pool of atherogenic LDL via accelerated transfer of LDL from plasma compartment, therefore, it increases the deposition of LDL cholesterol in the arterial wall (Meenakshisundaram *et al.*, 2010). Cigarette smoking increases plasma catecholamine which induces lipolysis and release of free fatty acids (Rashan *et al.*, 2016). HDL can have a protective role and inhibit the cholesterol and oxidized LDL damaging effects (Garrido *et al.*, 2000). After oral administration of aqueous bark extract of *Terminalia arjuna* the serum lipid profile viz., serum cholesterol, serum triglyceride, LDL and VLDL significantly decreases with increase in HDL level. *Terminalia arjuna* is a large tropical woody tree distributed throughout subtropical regions of India. It has free radicals scavenging property and antioxidant activity (Chatha *et al.*, 2014). Aqueous bark extract of *Terminalia arjuna* contains tannins, coumarins and terpenoids and revealed a high amount of polyphenol which may be responsible for antioxidant activity (Dutta, 2013). It exhibited a strong hydroxyl radical as well as superoxide anion radical scavenging potential, hence acting as an antioxidant (Ghosh *et al.*, 2013). The aqueous extract of *Terminalia arjuna* inhibited lipid peroxidation (LPO) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Kokkiripati *et al.*, 2013). Aqueous bark extract of *Terminalia arjuna* acts as a hypolipidemic, hypocholesterolemic and oxidative stress lowering agent (Sharma *et al.*, 2012).

References

- Carvalho, C.A.F., Favaro, W.J., Padovani, C.R. & Cagnon, V.H.A. (2006). Morphometric and ultrastructure features of the ventral prostate of rats (*Rattus norvegicus*) submitted to long-term nicotine treatment. *Andrologia*, 38(4): 142–151. doi: 10.1111/j.1439-0272.2006.00728.x.
- Chatha, S.A.S., Hussain, A.I., Asad, R., Majeed, M. & Aslam, N. (2014). Bioactive Components and Antioxidant Properties of *Terminalia arjuna* L. Extracts. *Journal of Food Processing & Technology*, 5(2): 1–5. doi: 10.4172/2157-7110.1000298.
- Dutta, M., Ghosh, A.K., Basu, A., Bandyopadhyay, D. & Chattopadhyay, A. (2013). Protective effect of aqueous bark extract of *Terminalia arjuna* against copper-ascorbate induced oxidative stress *in vitro* in goat heart mitochondria. *Int. J. Pharm. Pharm. Sci.*, 5(2): 439–447.
- Dwivedi, S. (2007). *Terminalia arjuna* Wight & Arn.-a useful drug for cardiovascular disorders. *J. Ethnopharmacol.*, 114(2): 114–129. doi: 10.1016/j.jep.2007.08.003
- Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, 18(6): 499–502.
- Garrido, R., Malecki, A., Hennig, B. & Toborek, M. (2000). Nicotine attenuates arachidonic acid-induced neurotoxicity in cultured spinal cord neurons. *Brain Res.*, 861(1): 59–68.
- Ghosh, A.K., Mitra, E., Dutta, M., Mukherjee, D., Basu, A., Firdaus, S.B., Bandyopadhyay, D., Chattopadhyay, A. (2013). Protective effect of aqueous bark extract of *Terminalia arjuna* on Cu²⁺-ascorbate induced oxidative stress *in vitro*: involvement of antioxidant mechanism(s). *Asian J. Pharm. Clin. Res.*, 6(Suppl.-1): 196–200.
- Hecht, S.S. (2002). Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. *Lancet Oncol.*, 3(8): 461–469.
- Ignácio, S.R., Ferreira, J.L., Almeida, M.B. & Kubelka, C.F. (2001). Nitric oxide production by murine peritoneal macrophages *in vitro* and *in vivo* treated with *Phyllanthus tenellus* extracts. *J. Ethnopharmacol.*, 74(2): 181–187.
- Kokkiripati, P.K., Kamsala, R.V., Bashyam, L., Manthapuram, N., Bitla, P., Peddada, V., Raghavendra, A.S. & Tetali, S.D. (2013). Stem-bark of *Terminalia arjuna* attenuates human monocytic (THP-1) and aortic endothelial cell activation. *J. Ethnopharmacol.*, 146(2): 456–464. doi: 10.1016/j.jep.2012.12.050.
- Meenakshisundaram, R., Rajendiran, C. & Thirumalaikolundusubramanian, P. (2010). Lipid and lipoprotein profiles among middle aged male

- smokers: a study from southern India. *Tob. Induc. Dis.*, 8(1): 11. doi: 10.1186/1617-9625-8-11.
- [12]. Patel, K., Prajapati, P., Sanghavi, S. & Goplani, V. (2014). A Study on Effects of Cigarette Smoking on Blood Cholesterol in Young Population of Ahmedabad. *International Journal of Basic & Applied Physiology*, 3(1): 106-110.
- [13]. Prakash, D., Suri, S., Upadhyay, G. & Singh, B. N. (2007). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. *Int. J. Food Sci. Nutr.*, 58(1): 18–28.
- [14]. Rashan, M.A.A., Dawood, O.T., Razzaq, H.A.A. & Hassali, M.A. (2016). The Impact of Cigarette Smoking on Lipid Profile among Iraqi Smokers. *International Journal of Collaborative Research on Internal Medicine and Public Health*, 8(8): 491–500.
- [15]. Reaven, G. & Tsao, P.S. (2003). Insulin resistance and compensatory hyperinsulinemia: the key player between cigarette smoking and cardiovascular disease? *J. Am. Coll. Cardiol.*, 41(6): 1044–1047.
- [16]. Roeschlau, P., Bernt, E. & Gruber, W. (1974). Enzymatic determination of total cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.*, 12(5): 226.
- [17]. Schettler, G. & Nussel, E. (1975). Determination of triglycerides ARB. *Med. SO2 Med. Prav. Med.*, 10: 25–8.
- [18]. Sharma, S., Sharma, D. & Agarwal, N. (2012). Diminishing effect of arjuna tree (*Terminalia arjuna*) bark on the lipid and oxidative stress status of high fat high cholesterol fed rats and development of certain dietary recipes containing the tree bark for human consumption. *Research in Pharmacy*, 2(4): 22-30.
- [19]. Singh, U.P., Singh, D.P., Maurya, S., Maheshwari, R., Singh, M., Dubey, R.S. & Singh, R.B. (2004). Investigation on the phenolics of some spices having pharmacotherapeutic properties. *J. Herb. Pharmacother.*, 4(4): 27–42.
- [20]. Soleimani, E. & Rahmani Kahnamoiei, J. (2014). The effect of the lateral cigarette smoke on serum lipid profiles in diabetic rats. *Cibtech Journal of Zoology*, 3(3): 116-119.
- [21]. WHO (2008). Report on global tobacco epidemic. The MPOWER package. Geneva: World Health Organization, ISBN 978-92-4-15968-2.
- [22]. Wybenga, D.R., Pileggi, V.J., Dirstine, P.H. & Di Giorgio, J. (1970). Direct manual determination of serum total cholesterol with a single stable reagent. *Clin. Chem.*, 16(12): 980–984.