Evaluation of phytochemicals from *Morinda citrifolia* (Noni) fruit extracts against nicotine-induced different physiological functions: An experimental study on albino rat model

Chaitali Kundu, Sandip K. Sinha*

Abstract

Background: People are consuming nicotine in the form of cigars, pipes and, cigarettes etc. and they are suffering from lungs, liver, heart diseases, involving hidden killer for human health. The *Morinda citrifolia* (Noni) is an important medicinal plant of the Rubiaceae family, commonly known as Ach, Bartundi, Hurdi and Sarangi in Bengali. Traditionally it is used in various purposes of food and medicines.

Aims and objectives: The present study was undertaken to highlight and find out the different phytochemicals from noni fruit extract and its role against nicotine-induced cardiac damage, mainly serum lipid profile and hematological changes and development of bio-based anti-nicotinic agent is now become an active research goal.

Materials and methods: Fruits of the selected plant was collected and prepared methanolic fruit extract. Fruit of *M. citrifolia* (Noni) contains several nutrients and phytochemicals. GC-MS analyzed the methanolic fruit extract of *M. citrifolia* to determine chemical constituents. The noni fruit extract was applied against the rat model's nicotine-induced blood parameters.

Results and discussion: Hematological and Biochemical alteration of cardiovascular experiments was done in albino rat. This study revealed that the fruits extract of *M. citrifolia* may be capable of reducing the cholesterol and hemoglobin levels significantly ($p<0.05$) in blood after nicotine treatment in rat models.

Conclusion: So, we try to find out the role of active principles and phytochemicals from this plant to combat the nicotinic activity. Results show that Noni fruit methanolic extract may have some protective effects against nicotine and its action.

Keywords: Nicotine, *Morinda citrifolia* (Noni), GC-MS, Biochemical and hematological study.

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Introduction

Divergent types of smoking are major hidden killers for human health. Smoking is responsible for killing of smokers and 50% chance of individual types of smoking-related death. Globally as well as the peoples of the 'Jangal-Mahal' area of West Bengal in India, are addicted to smoking and tobacco. People of different socio-economic group are addicted to smoking for lots of causes, thus result, they suffer from various health hazards. There are 7000 types of compound are present in tobacco smoke. Phenol, nitrosamines, and nicotine compound are of them. Some of them are carcinogenic; nicotine is a vasoactive compound that increases vascular resistance. Smoking rises heart rate and blood pressure within ten minutes of exposure. More than 35% of adults use tobacco in India, out of them most common form is beedis (53%) and cigarettes (19%). Several community-based surveys revealed that between never smokers and beedis smoking person are risk with coronary heart disease (CHD). Those consuming 25 beedis per day having a 10-fold increase risk.[1]

Nicotine is a strong alkaloid, was first extracted from tobacco by German physicians Wilhelm Heinrich Posselt and Kari Luwig Reimann it causes increase in heart rate, blood pressure and cardio contractility of humans.[2] So many people are consuming nicotine in the form of cigars, pipes, cigarettes, etc.; as a result, they suffer from various diseases involving major public health problems, including diseases of lungs, liver, heart, etc.[3]

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India is one of the richest countries in the world in terms of biodiversity, has 15 agro-climatic zones. Out of the 17000-18000 species of flowering plants, more than 7000 are estimated to have medicinal usage in folk and documented systems of medicine like Ayurveda, Unani, Siddha & Homoeopathy (AYUSH System of Medicine). In our Hindu culture "Rig-Veda" it is found that people use the herbal plant as folk medicine since our civilization. These medicinal plants also have good economic importance throughout the world. "Let food be your medicine and let medicine be your food" was advised by the father of medicine Hippocrates, over two millennia ago. It has seen that folk medicine have long history since ancestors and use this therapeutic agent during their struggles against different diseases and natural climates.[4]

According to the World Health Organization, the traditional medicine system will continue to play an essential role in the health care system since over 80 % of the population in the third world country relies on traditional medicine. Different medicinal plants have a long history use as traditional medicine.[5]

The *Morinda citrifolia* (Noni) is an important medicinal plant of the Rubiaceae family, commonly known as Ach, Bartundi, Hurdi and Surangi in Bengali. Traditionally used this medicinal plant for hypertension (from extracts of leaves and fruit), boils and carbuncles, stomach ulcers, jaundice, tuberculosis, fever, mouth and gum infection, diabetes, loss of appetite. At the same time it is also used for human vitamin A deficiency (leaves), attention disorder, addiction, cardiovascular diseases, chemical sensitivity, immune deficiency, inflammation, brain problems.[6] Leaf and fruit of MC contain several nutrients and phytochemicals are comprises common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins etc.[7] It has been reported that from the different parts of *M. citrifolia* identified more than 160 phytoconstituents. Out of them, 120 have biological activities.[8] Quercetin (anti-inflammatory and antioxidant activity)9 Scopoletin and Rutin, (anti- psychotic like activity),10 Xeronine (anticancer activity) are bioactive compound from noni fruits.[11] MC has been recognized as an important plant, for treating several physiological hazards worldwide. Nearly 120 bioactive phytoconstituents have been reported from Noni and variety micronutrients are produced after fermentation of the fruit extract.[12]

The present study was undertaken to highlight and find out the different phytochemicals (through GC-MS) from noni fruit extract as a bio-based anti-nicotinic agent and its role against nicotine-induced cardiac damages mainly serum lipid profile and hematological changes. Along with this, effort has been made to observe the cardiac tissue damage due to nicotine and recovery of those with Noni fruit extract through histological studies.

Methods and Materials

Plant materials collection

The medicinal plant *M. citrifolia* fruits are collected (May to June) from Bishnupur forest area of "Jungle Mahal" of Bankura district, West Bengal, India. The fresh ripe fruit were collected, washed under tap water, and shaded dried for a few days. The dried fruit materials were then prepared as dust form by grinder machine.[13,14] This plant was previously identified through Botany Department. (Ref. No. Bot. /Idn. Taxn/AKM-2022).

Preparation of Methanolic extract of the proposed plant

The fruit extract of methanol of the selected plant was prepared by using of cold extraction with sonication.[14] Five grams of dried power derived from the plant materials was mixed with 50 mL of 100% methanol with the aid of a magnetic stirrer at room temperature for 24 hours. The Methanolic extract of *M. citrifolia* (noni fruit) was prepared using shakers for 72 hours, and then the solution was filtered and evaporated under a vacuum in a rotary evaporator to obtain a dry mass extract. After filtration with filter paper, the solvent was removed with the utilization of a rotary evaporator. The dried solvent-free crude of noni fruit was stored in a 40°C temperature in airtight container until further use. The extract was weighed to 0.1 gm and dissolved in 1-mL of 0.9% NaCl solution.

Experimental animal maintenance and its care

Eighteen Wister strain male albino rats (n=6) obtained from SCLAB (State Centre for Laboratory Animal Breeding), West Bengal Livestock Development Corporation Limited (A Govt. of West Bengal Undertaken) Animal Resources Development Department, LB-2, Sector-III, Salt Lake City, Kolkata-6, Email: info@wbldc.in of weighing 110–120 gm were used. The rats were placed in a room with controlled cycles of 12 hours of light and 12 hours of darkness; during experiments, animal feeds adjusted to constant levels of fat, protein, vitamins, minerals and water were provided to the animal's *ad libitum*. All the rats were divided into three (03) groups: The control group with rodent food, Nicotine drug-treated group, and nicotine with plant extract-treated group. The total duration of experiment was 14 days.[13] Experiments were conducted at Vidyasagar University accepted principles of laboratory animal house (registration No. 2013/GO/Re/S/18/CPCSEA/2018 and our animal ethical Approval no. is VU/IAEC-I/SKS-1/3-8/19, dated 11.12.19).

Preparation of Nicotine solution

In male albino rat model the nicotine drugs was applied on the basis of (3.5 mg/kg body weight) for 14 days. The nicotine solution is highly concentrated so it is diluted as per body weight of rat. In this animal model try to apply the drugs on the basis of their body weight because this dose is equal to find in case of both male and female albino rat.[15]

Division of group of experimental animals

Group I: Control group with rodent food
Group II: Nicotine drug-treated group.[15]
Group III: Treated with nicotine and plant extract orally inject on the basis of 500 mg /kg body weight for 14 days.[13]

Hematological study

The blood was collected from animals by heart punctures. Then blood hemoglobin level, RBC, WBC and platelets counts were measured.[16]

Biochemical studies for estimation of lipid profile level

For the determination of serum cholesterol, triglyceride, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) kit method was adopted. For this method test sample (serum), standard and blank were pipetted using a micropipette to

the ependrof tube. The concentration of absorbance was calculated from the slope concentration chart of standard.[17,18]

Statistical Analysis

All data are expressed as Mean ± Standard Error of Mean (SEM) with "student t-test ". One-way ANOVA will analyse data to compare the difference in means of more than two groups.[19]

Results

GC-MS analysis

GC-MS analysis of the Methanolic extracts of fruit parts was performed using a GC-MS (GC Trace GC ultra, MS-POLARISQ, Thermo Scientific India Pvt. Ltd) autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a capillary column (TR-WAXMS,30m × 0.25 mm [ID] × 0.25 µm film thickness). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70eV with scan range 50–750 a.m.u (mass ratio). Helium gas (99.999%) was used as carrier gas at a constant flow rate of 0.9 µL/min and an injection volume of 1-µL was employed (split ratio of 18:1). The injector temperature was maintained at 260°C. The ion source temperature was 230°C. The oven temperature was programmed from 70°C (Isothermal for 2 minutes), with an increase of 20°C / min. to 110°C, then 5°C / min. to 200°C, ending with a 10 minute isothermal at 260°C. Mass spectra were taken at 70 eV; full scan mode and fragments from 50–750 amu. The solvent delay was 0 to 2:00 minutes and the total GC-MS running time was 31 minutes.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The Mass detector used in this analysis was TQ Quadruple Mass Spectrometer and the software adopted to handle mass spectra and chromatograms was a MS Work station.[20,14]

Chromatogram for GC-MS analysis: In our nature, various medicinal plants are available worldwide and have so many nutrients. Some of the phytochemicals substances like phenols and flavonoids are important substances responsible for several medicinal values like anticancer, antioxidant, antifungal, antibacterial and anti-psychotic activity. The identification of the phytocompounds was carried out based on the retention time and molecular formula.[7] Table 1 shows the GC-MS results of MC (Noni) fruits. The name of identified phytocompounds in the fruit of MC (Noni) with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area percentage were given in Table 1.

The results of the present study revealed that there are several phytocompounds shown in the methanol extracts of fruits of MC by GC-MS analysis. They are carrying out several biological activities. Noni fruits are good sources of ester, terpenoids etc, bioactive compounds. The manifestation of numerous bioactive compounds and their therapeutic confirmations rationalizes using this plant for curing different health-related problems.[21] In Figure 1 shows the result of chromatogram. The selected peaks are 12.44, 13.44, 16.79, 19.72, 23.03, 23.61, 25.48, 26.58, 28.50, 28.79, 30.09, 31.89 and 32.46.

If these Retention time (Minute) denotes different products, then the percentage study will be like this.12.44--1.27; 13.44--0.35; 15.38--0.18; 16.79--15.18; 18.81--0.31; 19.72--4.4; 21.82--0.14; 22.76--0.08; 23.03--1.40; 23.61--0.53; 24.13--0.40; 24.71--0.48; 24.90--0.56; 25.25--0.07; 25.48--1.97; 25.67--1.2; 26.37--0.02; 26.58--21.14; 27.48--0.31; 27.78--0.14; 28.50--44.65; 28.79--0.13 29.21--1.39; 29.89--0.18; 30.09--0.29; 31.89--3.07.

Haematological Study

Combined effects of Noni fruit Methanolic extract and nicotine on hematological parameters

From Figure 2 and Table 2, it has been seen that the percentage of Hb (Haemoglobin) level was significantly ($p<0.05$) decreased in nicotine-induced group (II) compared to control (Group -I). On the other side for hematological analysis in the nicotine-treated group, the RBC and platelet count are significantly ($p <0.05$) reduced towards the control group. But the WBC count was significantly ($p<0.05$) raised in nicotine-treated animal than a control group. At the time in another group (Group-III) the level of Hb, the amount of RBC, WBC and platelets are significantly ($p<0.05$) changed after treatment of MC (noni) fruit extract in nicotine pre-induced animals in compared to nicotine treated group (II) only.

Lipid profile test:

Combined effects of Noni fruits Methanolic extract and nicotine on lipid profile parameters

Figure 3 and Table 2 shows that in nicotine treated group (II) the total serum cholesterol (TC) level was higher than control group (I). But after the injection of Noni fruit extract in a nicotine-treated animal, the cholesterol level declined significantly ($p<0.05$). On the other hand, the level of blood Triglyceride (TG) in nicotine treated group was increased compared to control group (I) and after treatment of fruit extract it was decreased significantly ($p<0.05$). Where as in nicotine-induced group the low-density lipoprotein [LDL]

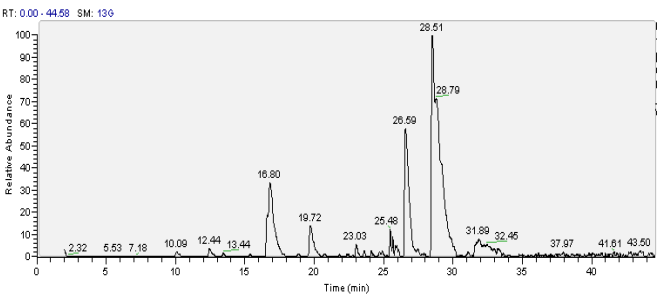

Figure 1: Chromatogram for GC-MS analysis of methanolic fruit extract of *Morinda citrifolia* (Noni).

Table. 1: Name of identified phytocompounds in methanolic extract of fruit of Morinda citrifolia by GC-MS analysis

Name of compound	*Rt*	*M.F*	*M.W*	*Peak area %*	*Nature of compound*	**Activity*
4-Pyrimidinamine, 6 methyl-	12.44	$C_5H_7N_3$	109	1.08	Pyrimidine derivative	Catechol-o-methyl transferase inhibitor
Hydrazinecarbothioamide	13.44	CH_5N_3S	91	0.3	Amide	
2-Propenal,3-(dimethylamino)-2-(methylamino)	15.38	$C_6H_{12}N_2O$	128	0.16		
3-n-Butylthiolane	16.79	$C_8H_{16}S$	144	12.82		Anti-tumour, Nicotinlytic, increase NK cell activity
4-Aminopyrimidine	18.81	$C_4H_5N_3$	95	0.27	Pyrimidine derivative	
4-Ethyl-2-hydroxycyclopent-2-en-1-one	19.72	$C_7H_{10}O_2$	126	3.73	Pyran ester	Decrease endothelial platelet adhesion.
2-Methyl-3pentyn-2-ol	21.82	$C_6H_{10}O$	98	0.12		Catechol-o-methyl transferase inhibitor
3-Hexanol,2-methyl	22.76	$C_7H_{16}O$	116	0.07	Alcohol	Methyl Donor
4(1H)-Pyrimidinone,6-methyl	23.03	$C_5H_6N_2O$	110	1.19	Pyrimidine derivative	HDL genic suppresses HMG-CoA reductase activity.
1,3-Dioxolane,2-methyl-2-pentyl	23.61	$C_9H_{18}O_2$	158	0.45		Catechol-o-methyl transferase inhibitor
3-(Benzylmethylamino-)-1-propanol	24.13	$C_{11}H_{17}NO$	179	0.34		
Phthalic acid, 2-ethoxyethyl propyl ester	24.71	$C_{15}H_{20}O_5$	280	0.41	Phthalic acid di-ester	Increase aromatic amino acid decarboxylase activity.
Propanoic acid, 3-chloro-,methyl ester	24.90	$C_4H_7ClO_2$	122	0.48		Increase aromatic amino acid decarboxylase activity.
2,4,7-Trioxabicyclo(4,4,0) dec-9-ene,8-decyloxy-3-phenyl	25.25	$C_{23}H_{34}O_4$	374	0.06		Decrease NE production
Cyclohexanecarboxaldehyde,4-(hydroxymethyl)	25.48	$C_8H_{14}O_2$	142	1.67		
Phthalic acid, pentyl tridec-2-yn-1-yl ester	25.67	$C_{26}H_{38}O_4$	414	1.02	Phthalic acid di-ester	Uric acid inhibitor
10-Pentadecen-5-yn-1-ol(E)	25.85	$C_{15}H_{26}O$	222	1.63		Erythro-cytogenic, ionic channel opener.
1,2,5-Oxadiazol-3-amine,4-(phenylmethoxy)	26.37	$C_9H_9N_3O_2$	191	0.02		
Dodecanoic acid,2-methyl-	26.58	$C_{13}H_{26}O_2$	214	17.86		Methyl Donor
Benzene,[2-methyl-1-(1-methyl ethyl)propyl]	27.48	$C_{13}H_{20}$	176	0.27		Methyl Donor
1,3-Dioxolane,2-methyl-2-(phenylmethyl)	27.78	$C_{11}H_{14}O_2$	178	0.12		Methyl-Guanidine inhibitor
Undec-10-ynoic acid,butyl ester	28.50	$C_{15}H_{26}O_2$	238	37.71		Inhibit uric acid production.
(Z)6,(Z)9-Pentadecandien-1-ol	28.79	$C_{15}H_{28}O$	224	0.11		Provide Zinc
1-Cyclohexyl-1-pentyne	29.21	$C_{11}H_{18}$	150	1.18		
2-Butene,1-bromo-2-chloro	29.89	C_4H_6BrCl	168	0.16		
18-18-Bi 1,4,7,10,13,16-hexaoxacyclononadecane	30.09	$C_{26}H_{50}O_{12}$	554	0.25	Crown ether	Increase Vita-D, K bioavailability, Bilirubinolytic.
Isobutyl 2,5,8,11-tetraoxatridecan-13-yl carbonate	31.89	$C_{14}H_{28}O_7$	308	2.6		Antioxidant

*Dr. Duke's phytochemicals and ethnobotanical database.

was significantly higher than the control group, but after treatment of M. citrifolia (MC) fruit extract the level of LDL and VLDL is significantly ($p<0.05$) reduced. The high-density lipoprotein (HDL) was less in nicotine treated group with compared to control group. Which was nearly same as the control group in noni fruit extract-treated group (III).

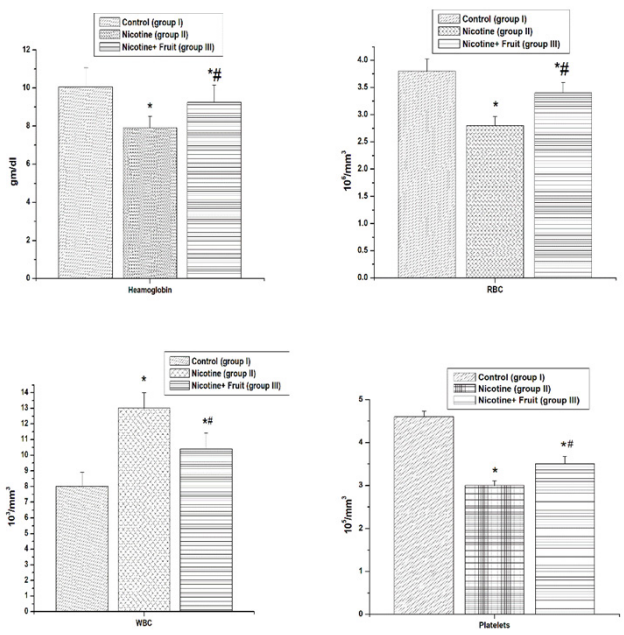

Figure 2: The histograms were representing the changes of haematological parameters of different groups. Here * indicate the level of significance (at P < 0.05) difference by One-way ANOVA in comparison to the group I (control group) with other groups. # Indicate significant changes of group II (nicotine treated group) with group III (nicotine and noni fruit extract treated group).

Histological observation

After sacrificed the treated animals, cardiac tissues was collected and fixed in formalin. Then the section was prepared for microscopic study as follows: The heart tissues were fixed in neutral formaldehyde (10%) solution, embedded in paraffin, and processed into 5 µm sections for light microscopy according to routine procedures. The paraffin section was stained with hematoxylin and eosin method.[22] Figure 4 (a-c) shows the histological changes of cardiac tissue in control, nicotine treated and nicotine plus

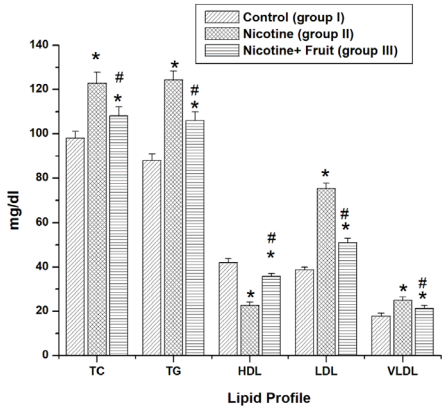

Figure 3: The diagrams were representing the changes of serum lipid profile of different groups. Here * indicate the level of significance (at P < 0.05) difference by One-way ANOVA in comparison to the group I (control group) with other groups. # Indicate significant changes of group II (nicotine treated group) with group III (nicotine and noni fruit extract treated group).

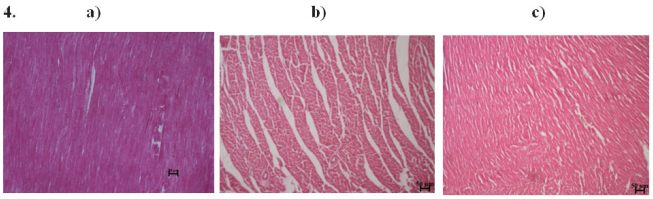

Figure 4: a) Control (Group-I), b) Nicotine treated (Group II), c) Nicotine + Noni fruit extract (Group III).
Figure a) Indicate histological section of heart tissue in control group (I). Figure b) Indicate heart tissue of nicotine treated group (II) in damage condition, Figure c) Denotes heart tissue of nicotine and MC fruit treated group (III) in recovery in comparisions group I.

Noni fruit extract, respectively. In Figure 4 (b) it was observed that perfect damage (cardiac tissue becoming fragmented) of heart tissue in case of nicotine treated animal (group II), whereas in figure-4 (c) showed the effects of nicotine-treated and Noni fruit extract (group III) that damages of heart tissue become recovered and similar to control (group I).

General Discussion

From an overview of the present study, the GC-MS analysis of the methanolic extract of MC fruits exposed the presence of several bioactive compounds. Their chemical structures are given in Table 1. From the previous studies of this plant, many extractions have been done by different solvents. Many research articles already reported that various types of phytocompounds are found from noni plant (fruit, leaves, and root) in the form of different chemical solvent. *M. citrifolia* has been considered as useful therapeutic medicinal plant against cardiovascular diseases, particularly hypertension,[23] atherosclerosis and dyslipidemia.[24] MC (Noni) has a positive and significant compound for reducing cholesterol biosynthesis by inhibiting HMG Co-A. This enzyme plays a key role in controlling lipid levels in plasma and other tissue.[13]

From our methanolic extraction of noni fruit showed twenty seven phytocompounds such as 4-Pyrimidinamine, 6methyl-(1.08%), Hydrazinecarbothioamide (0.3), 2-Propenal,3-(dimethylamino)-2-methylamino (0.16%), 3-n-Butylthiolane (12.82%), 4-Aminopyrimidine (0.27%), 4-Ethyl-2-hydroxycyclopent-2-en-1-one (3.73%), 2-Methyl-3pentyn-2-ol (0.12%), 3-Hexanol,2-methyl (0.07%), 4(1H)-Pyrimidinone,6-methyl (1.19%), 1,3-Dioxolane,2-methyl-2-pentyl (0.45%), 3(Benzylmethylamino-)-1-propanol (0.34%), Phthalic acid, 2-ethoxyethyl propyl ester (0.41%), Propanoic acid, 3-chloro- methyl ester (0.48%), 2,4,7-Trioxabicyclo (4,4,0) dec-9-ene,8-decyloxy-3-phenyl (0.06%), Cyclohexanecarboxaldehyde,4-(hydroxymethyl) (1.67%), Phthalic acid, pentyltridec-2-yn-1-ylester (1.02%), 10-Pentadecen-5-yn-1-ol(E) (1.63%), 1,2,5-Oxadiazol-3amine,4(phyenylmethoxy) (0.02%), Dodecanoic acid,2-methyl- (17.86%), Benzene,[2-methyl-1 (1-methyl ethyl) propyl] (0.27%), 1,3-Dioxolane,2-methyl-2-(phenylmethyl) (0.12%), Undec-10-ynoic acid, butyl ester (37.71%), (Z)6,(Z)9-Pentadecandien-1-ol (0.11%), 1-Cyclohexyl-1-pentyne (1.18%), 2-Butene,1-bromo-2 chloro (0.16%), 18-18 Bi1,

Table. 2: Biochemical parameter of methanol extracts of *Morinda citrifolia* fruits against nicotine treated animal

Parameter		*Control*	*Nicotine*	*Nicotine ± Fruit extracts*
Haematological Test	Hb (gm/dl of blood)	10.06 ± 0.79	7.9 ± 0.27 *	9.24 ± 0.25 *#
	RBC (10^6 cumm)	3.8 ± 0.63	2.8 ± 0.96 *	3.4 ± 0.37 *#
	WBC (10^3 cumm)	8.0 ± 0.46	13 ± 1.1 *	10.4 ± o.27 *#
	Platelet(10^5cumm)	4.6 ± 0.14	3 ± 0.11 *	3.5 ± 0.17 *#
Lipid profile Test	Total cholesterol (mg/dl of serum)	98.11 ± 2.2	122.89 ± 1.2*	108.11 ± 1.8 *#
	TG (mg/dl of serum)	87.99 ± 2.4	124.39 ± 3.1 *	106 ± 2.1 *#
	HDL (mg/dl of serum)	41.88 ± 1.9	22.68 ± 0.9 *	35.79 ± 1.5 *#
	LDL (mg/dl of serum)	3.7 ± 2.2	75.33 ± 1.4 *	51.04 ± 2.3 *#
	VLDL (mg/dl of serum)	17.77 ± 1.4	24.88 ± 1.6 *	21.27 ± 1.4 *#

All the data are expressed in terms of MEAN ± SEM. Statistical analysis was done by One-way ANOVA. Here * indicate the level of significance at P < 0.05 in between the group-I (Control group) with other two groups and # indicate the level of significance at P<0.05 in between group II (Nicotine treated group) and group III (Nicotine and Noni fruit extract).

4,7,10,13,16hexaoxacyclononadecane (0.25%), Isobutyl 2,5,8,11-tetraoxatridecan-13-yl carbonate (2.6%).[25]

Due to the different climatic conditions and nature of the soil in our Jungalmahal area, the above-mentioned compounds are different from others, which are already reported. Tridecaethylene glycol monomethyl ether, acetate, Methyl 11-methyl-dodecanoate, Cyclohexene,1-ethenyl-1methyl2,4bis [1methylethenyl] [1S(1a',2a',4a')]-, Hexadecanoic acid,2-ethyl methyl ester, Propionic acid, 3-(allythio)-,ethyl ester phytocompounds are found from noni leaf andHydrazinecarbothioamide,2Propenal,3(dimethylamino)2methylamino,3nButylthiolane,4 (1H)Pyrimidinone,6methyl, Phthalicacid, pentyltridec2yn1ylester,(Z)6, (Z)9Pentadecandien1ol, Isobutyl2,5,8,11-tetraoxatridecan-13-ylcarbonate are found from noni fruits, which are more active and they have some beneficial effect against different diseases.

From our GC-MS analysis (shown in Table-1) of methanolic Noni fruit extract the different types of new phytocompounds are analyzed, which are not reported in any previous study. These new phytocompounds may have some important medicinal characteristics. Probably they have some active bioactivity nature. Noni fruit also contains Scopoletin and alkaloids. Scopoletin produces serotonin, a chemical compound that blocks the work of the smooth muscles and nerves and causes vasoconstriction of the heart and brain membranes.[26] So, Noni fruit extract may be useful as an important medicinal phytocompounds applicable in pharmaceutical industries for drug development against cardiac diseases and could be valuable as an effective drug in medicine for maintaining good health.[27]

It is well known that smoking induces a high level of endogenous lipid production. A different study also reported that Noni's ripe fruit extract significantly reduces the lipid level. In this reducing mechanism, lipid level may be involved through the inhibition of cholesterol biosynthesis and the inhibition HMG Co-A. This enzyme may play key role in controlling lipid levels in plasma and other tissue.[28,29] From the hematological study (Figure 2 and Table 2) it was observed that Hb level, RBC, WBC, and platelets count are significantly changed in (p<0.05) compared to control group, nicotine treated group and nicotine plus MC fruit extract treated group. The fruit extract content of the protein, carbohydrates and vitamin C will increase in number along with the increase in the fruit's maturity. As we know, the role of vitamin C is to help in the absorption of iron so it can be absorbed easily by the body, thereby increasing the number of red blood cells in the body. According to a previous study, vitamin A is needed in several essential processes in the body such as metabolism, blood cell formation, the regulation of cell differentiation and the immune system.[30]

Several present investigations also reported that Noni possesses antioxidant, antimicrobial, antifungal, antiangiogenic, antidyslipidemic, hypoglycaemic, hepatoprotective activity, and immunomodulatory properties.[31] Different types of other research revealed that traditional uses of Noni extract are to relieve sore throat, carbuncle, peeling or cracking of the toes and feet, treating stomach ulcer, and hypertension.[32] With this above information, we tried to determine the serum total cholesterol (TC), triglyceride (TG) level, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) level, very low-density lipoprotein (VLDL) by kit method. In this study we used different animal models to evaluate the possible mode of action of the anti-nicotinic effect of noni fruit extract. Figure 3 and Table 2 shows the lipid profile test in control group, nicotine-treated group and nicotine plus MC fruit extract-treated group. TC, TG, HDL, LDL, VLDL level was significantly changed in 0.05 level. Noni plant is abundant with flavones, may have some inhibitory role regarding lipid biosynthesis. At the same time through the histological studies in figure-4 of heart tissue, it was found that a distinct damages of heart tissue in case of nicotine-treated animal (group II), whereas in case of combined exposure of nicotine (treated) and noni fruit extract (group III) that damages of heart tissue become recovered and similar to control group (I).

This study revealed that the plant *M. citrifolia* (fruit extract) may be capable to reduce the cholesterol and hemoglobin level in blood after nicotine treatment in rat models. This

investigation may benefit common and poor people (Jangal Mahal area) to protect against cardiac disorders. Our work GC-MS analysis of Noni methanolic fruits extract shows that there are so many new phytochemicals (Table-1) present in this plant, and they may have some different potential biochemical roles for the recovery of cardiac health. Further studies are also required in future to isolate the specific active components of fruit extract of *M. citrifolia*, which may be applicable for drug development for anti-nicotinic activity on cardiovascular health.

All these findings may be more beneficial for our tobacco-addicted community people to reduce the health impact of nicotine-related cardiovascular diseases. The results from this study justify the medicinal use of *M. citrifolia* fruit to reduce the cholesterol, and triglyceride level in blood, and recovery of tobacco-induced cardiac tissue damage.

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Conflict of Interest

The authors declare that, they have no conflict of interest.

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