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Evaluation of cardioprotective potential of aqueous extract of *Nigella Sativa* against Doxorubicin induced cardiotoxicity in Albino rats—An experimental study.

Ram Kishor Pandey[1], Monica Sharma[2], Pinki Vishwakarma[3], Raj Kumar Goel[4], Manish Saini[5]

[12345]Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut, U.P. India
Correspondence to—Ram Kishor Pandey, MD
Assistant Professor, Department of Pharmacology, L.L.R.M. Medical College, Meerut U.P.
E-Mail-rampandey4u@gmail.com

Abstract

Objectives*:* *Nigella sativa* also known as Kalonji, used in ethnomedicine for treatment of various cardiovascular disorders. current study is aimed to evaluate cardioprotective potential of aqueous extract of *Nigella sativa* (AENS) against Doxorubicin (DOX) induced cardiotoxicity.

Methods: Study was conducted in which 30 Wister albino rats were divided in 05 groups,06 rats in each group**. Group I-** Serve as control group received 2ml/kg body weight normal saline per os (p.o.) for 21 days**, Group-II** received pellet diet along with tap water as required for 21 days and injection DOX 20mg/kg was administered intraperitoneally (i. p.) on 21st day**, Group III and Group IV-** AENS 250+500mg/kg/day+ Injection DOX 20mg/kg i.p. on 21st day. **Group V**- Carvedilol 30 mg/kg/day p.o. for 21 days followed by Injection DOX 20 mg/kg i. p. single dose on 21st day, then all animals were anaesthetized by administering Ketamine and Diazepam i.p. cardioprotective potential of graded doses of AENS was evaluated by measuring serum level of CK-MB, LDH, SGOT, SGPT. After scarification of animal's histopathological examination (HPE) of heart was performed. Data were appropriately organized and analyzed by using ANOVA and Post Hoc test.

Results: Cardiac biomarkers CK-MB, LDH, SGOT, SGPT were raised remarkably in groups treated with DOX. AENS administered group revealed remarkable limitation in rise of CK-MB, LDH, SGOT, SGPT ($p<0.001$) in a dose dependent manner following administration of DOX which were comparable to the group treated with standard cardioprotective drug Carvedilol. histopathological changes were also corelated cardioprotective potential of *Nigella sativa*.

Conclusion: Graded doses of AENS revealed significant cardioprotective potential against DOX induced cardiotoxicity.

Key words—*Nigella sativa*, Carvedilol, Doxorubicin, Cardiotoxicity, Wister albino rats

Introduction

Nigella Sativa also known as black seed or black cumin, an annual herb having many ethnopharmacological properties. it is cultivated in many parts of the world like in South Europe, India, Pakistan, Turkey and in Saudi Arabia(1) it is an important drug in Indian traditional system of medicines(2)and is being used in Tibbs-e-Navi(Prophetic medicine)(3).many active compounds present in *Nigella sativa* like thymoquinone(TQ),alkaloids (Nigellicines and Nigellidine),saponins and flavonoids which has beneficial effects in treatment of various diseases including CVS

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diseases(4,5).many of the therapeutic properties of this plant is because of presence of Thymoquinone (TQ) (6) TQ has been associated with decrease in blood pressure by increasing urine output and reduction in oxidative stress by Ca+ channel blockade.(7),for doxorubicin induced cardiotoxicity the accepted mechanism is the formation of reactive oxygen species (ROS) creating oxidative stress**.** Tocopherols present in *Nigella sativa* has shown scavenging potentials of free radicals, which is supposed to terminate lipid peroxidation (8). Nigella sativa has many beneficial properties which includes its ability to enhance antioxidation (9), cardioprotective activities viz. antiplatelets, hypolipidemic and hypotensive action (10) DOX induced cardiotoxicity is a well-established standard model to study the beneficial effects of many ethnomedicines on cardiac dysfunction. very few studies assessing the cardioprotective potential of *Nigella sativa* are currently available therefore the present study was undertaken to investigate the cardioprotective potential of AENS.

Methods

Experimental Animals

Healthy albino Wister rats of either sex weighing 150g-250g were procured from CPCSEA approved central animal house of the LLRM Medical college, selected animals were grouped and housed in poly propylene cages and maintained under standard laboratory conditions of alternating periods of light and darkness of 12 hr. each, temp.25±2degree Celsius, relative humidity 45-50% with free access to standard rat pellet diet and tap water *ad libitum*.one week of acclimatization was allowed, thereafter they are included in study except pregnant rats. the dose *of Nigella sativa* extract used in the study were calculated on basis of previously documented LD50 on rats according to OECD guidelines (OECD_423)

Method of preparation of extract

Nigella sativa

The leaves of *nigella sativa* were washed and dried at room temperature for 2-3 days and then grinded to fine powder in mixer followed by extraction with 95% ethanol by using Soxhlet apparatus for 15 hours, after filtration, the filtrate was concentrated at 65 degree Celsius by using rotary evaporator**.** The concentrate was then freeze dried. Solution of dried leaves of *Nigella sativa* powder was prepared in distilled water and was used in study (11)

Materials

Commercially available injection Doxorubicin (Khandelwal Laboratories Pvt. Ltd.), tab Carvedilol 25 mg (Sun Pharmaceutical Industries Ltd.) was procured from market, and used in the study.

Study design

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This study was conducted in the department of Pharmacology, L.L.R.M. Medical College, Meerut, U.P. India

The animals were randomly divided into 5 groups of 6 animals each, the group were described as **Group-I:** Control group was given 0.9% normal saline in single oral dose of 2ml/kg body weight daily for 21 days

Group-II: pellet diet and tap water as desired, Doxorubicin in single dose of 20 mg/kg i. p. on 21st day.

Group-III and Group-IV: These groups were treated with AENS in 2 selected graded doses of 250 mg/kg/day and 500mg/kg/day orally for 21 days respectively followed by administration of Doxorubicin (20mg/kg i. p.) as in group-II

Group-V: Carvedilol (standard cardioprotective drug) 30mg/kg/day p.o. for 21 days then administration of Doxorubicin (20mg/kg i. p.) in single dose on 21st day.

Sample collection

After 48 hours of fasting of DOX administration, animals of all groups were anesthetized and sacrificed by using intraperitoneal Ketamine(75mg/kg) and Diazepam (10 mg/kg). blood sample was collected from abdominal aorta for performing blood tests i.e.CK-MB, LDH, SGOT, SGPT. Histopathological examination was performed on animals' heart.

Data thus obtained was appropriately organized and analyzed by ANOVA and Post Hoc test.

Estimation of Biochemical Parameters

Serum separation from collected blood sample was done by using Remi R-8 Centrifuge. Centrifuge speed maintained at about 2500rpm for 10 minutes then following tests were performed.

1-Creatinine Kinase MB fraction (CK-MB)

2- Lactate dehydrogenase (LDH)

3-Serum glutamate oxaloacetate transaminase (SGOT)

4-Serum glutamate pyruvate transaminase (SGPT)

Observation and Results

Mean ± SD was calculated for each group and ANOVA test was applied to test significance of results. P- values determined by given reference in appropriate table (12) p-values <0.05 were considered significant.

Biochemical parameters

1-CK-MB

The mean CK-MB level in normal saline treated group was 0.75 ±0.01 IU/L and It was found to be increased significantly after administration of Doxorubicin, administration of standard drug Carvedilol reduced significantly the rise in CK-MB levels after administration of Doxorubicin

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however with *Nigella sativa*, dose dependent limitation of CK-MB rise was observed following doxorubicin administration. when AENS was administered in dose of 250mg/kg p.o. for 21 days although significant limitation of CK-MB rise was observed when compared to Doxorubicin treated group but it does not match the efficacy of Carvedilol treated group. however, in dose of 500mg/kg p.o. for 21 days with AENS had better efficacy in limiting the CK-MB rise to following administration of Doxorubicin which was found to be statistically significant($p<0.001$) (Table 01, figure 01)

2-LDH

The mean LDH level in normal saline treated group was 245.16 ±5.37 IU/L. it was increased considerably following administration of Doxorubicin.

However, administration of standard drug Carvedilol limited the rise of LDH levels remarkably following administration of Doxorubicin.

AENS revealed dose dependent restriction of LDH rise after doxorubicin administration. although the dose of 250 mg/kg orally for 21 days showed significant limitation of LDH rise when compared to Doxorubicin treated group but it did not match the efficacy of Carvedilol treated group. however, in dose of 500 mg /kg p.o. for 21 days the AENS had much better efficacy as expected, in reducing the LDH rise following administration of Doxorubicin which was statistically significant ($p<0.001$) (Table 01, figure 01)

3-SGOT

The mean SGOT level in normal saline treated group was 34.25 ±0.60 IU/Land it was found to be increased considerably following administration of Doxorubicin. Administration of standard drug Carvedilol reduced remarkably the rise of SGOT levels following Doxorubicin administration. AENS revealed dose dependent restriction of rise in SGOT levels after Doxorubicin administration. Although the dose of 250mg/kg orally for 21 days demonstrated remarkable limitation of SGOT rise when compared to Doxorubicin treated group but it did not match the efficacy of Carvedilol treated group. However, in the dose of 500 mg/kg orally for 21 days the AENS showed much better efficacy in reducing the SGOT level following administration of Doxorubicin. (Table 01, figure 01)

4-SGPT

The mean SGPT level in normal saline treated group was 36.08 ±0.31 IU/L.it was increased considerably following administration of Doxorubicin, administration of standard drug Carvedilol reduced the rise of SGPT levels noticeably following administration of Doxorubicin. AENS in dose of 250mg/kg p.o. for 21 days revealed restriction of SGPT rise following administration of Doxorubicin and shown noticeable reduction in SGPT level but its efficacy of Carvedilol treated group was unremarkable. However, in dose of 500 mg/kg p.o. for 21 days the AENS showed much better efficacy in reducing the SGPT level following administration of Doxorubicin which was statistically significant ($p<0.001$) (Table 01)

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GROUP	TREATMENT	CK-MB	LDH	SGOT	SGPT
I	Normal saline(2ml)	0.75 ±0.01	245.16 ±5.37	34.25 ±0.60	36.08±0.31
II	Doxorubicin (20)	13.55 ±0.07	1335 ±20.74	217.66±9.17	171.33±2.11
III	Carvedilol (30)	2.5 ±0.16	505.16 ±7.77	115±1.57	75.66±1.3
IV	*Nigella sativa* (250)	9.76 ±0.13	804.83 ±4.40	174.5±4.57	106.5±1.86
V	*Nigella sativa* (500)	4.76 ±0.14	127.83 ±1.67	187.83±1.67	84.66±2.12

Table-01: Effect on biochemical parameters of Doxorubicin, Carvedilol, *Nigella sativa* 250, *Nigella sativa* 500 treated group compared with normal saline treated group (Mean ± SE, n=6) p<0.001

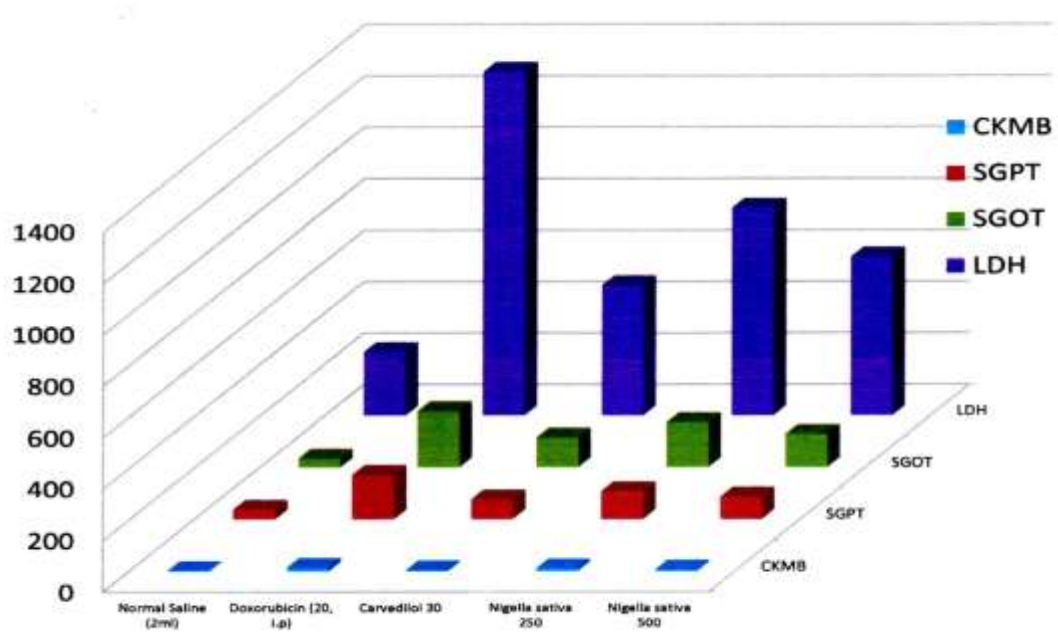

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Figure 01: Effect of Carvedilol and Aqueous extract of *Nigella sativa* in their respective doses against Doxorubicin induced changes in various biochemical parameters (Mean ±SE) (n=6) Histopathological changes

Cut section of cardiac tissue of different group of rat's hearts with cytological preparation stained with hematoxylin and eosin (H&E staining) seen under light microscope (magnified×45) histopathological examination (HPE) of heart of rats administered with normal saline showed normal striated muscles fibers with visible striations and well aligned oval flattened nuclei (fig-2) Doxorubicin administration resulted in intramuscular hemorrhage, fragmentation of muscles fibers and infiltration with inflammatory cells (fig-3) Rats administered with *Nigella sativa* (250mg/kg/day p.o.+ Doxorubicin 20mg/kg i. p. single dose) for 21 days showed derangement of muscles fibers and hemorrhage(fig-4). In rats treated with *Nigella sativa* (500mg/kg/day p.o.+ Doxorubicin 20mg/kg i. p. Single dose) for 21 days showed cytoplasmic eosinophilia, centrally placed nuclei in few muscles' fibers and almost normal cardiac architecture which was similar to that seen in Carvedilol treated groups (fig 5,6) degree of protection with *Nigella sativa* against Doxorubicin induced cardiotoxicity was evident clearly on histopathological examination of cardiac tissue.

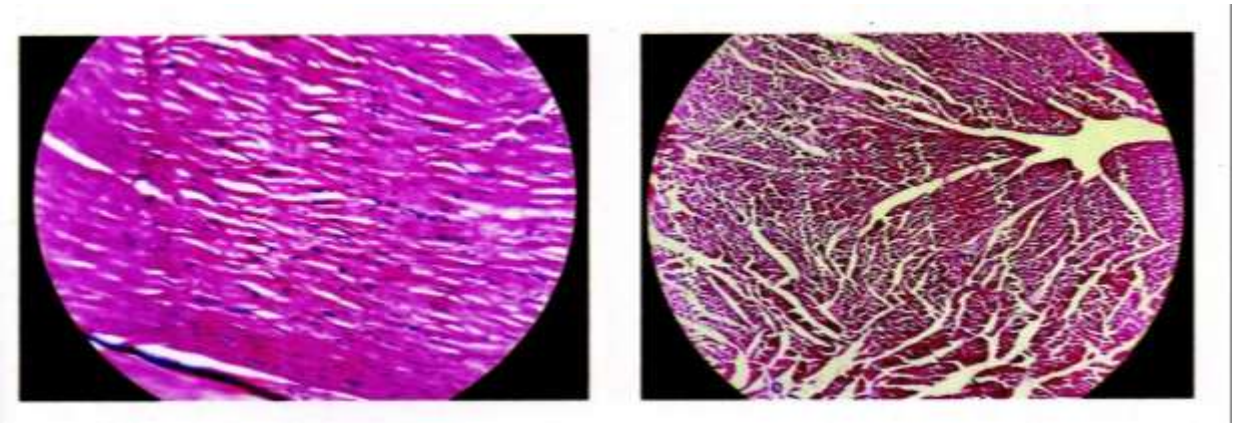

Figure 02: Normal histological features of heart of rats treated with normal saline

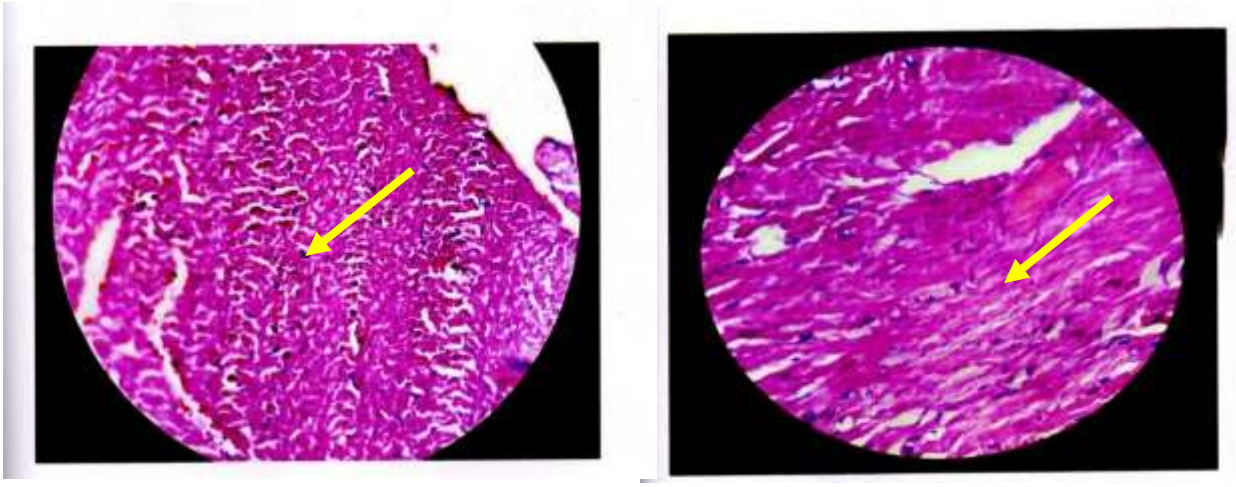

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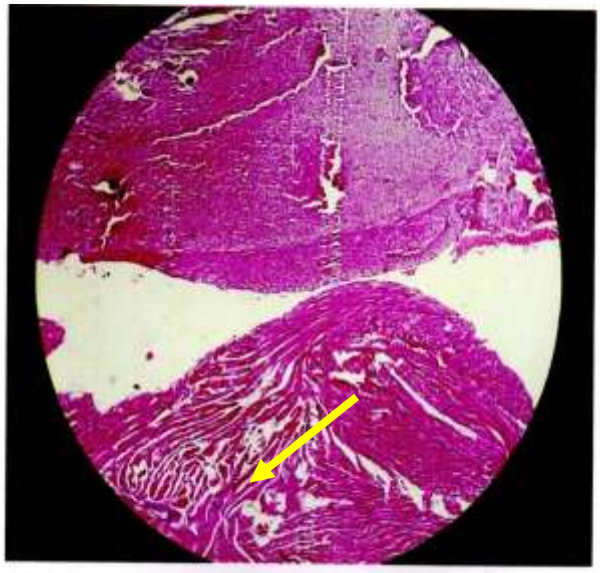
Figure 03: Histological features of the heart of the group treated with Doxorubicin showing intense inflammation, hemorrhage, degeneration and fragmentation of myocardial fibers (arrow mark)

Figure 04: Histological features of the heart of group treated with *Nigella sativa* 250mg + Doxorubicin showing derangement of muscle fibers and hemorrhage (arrow mark)



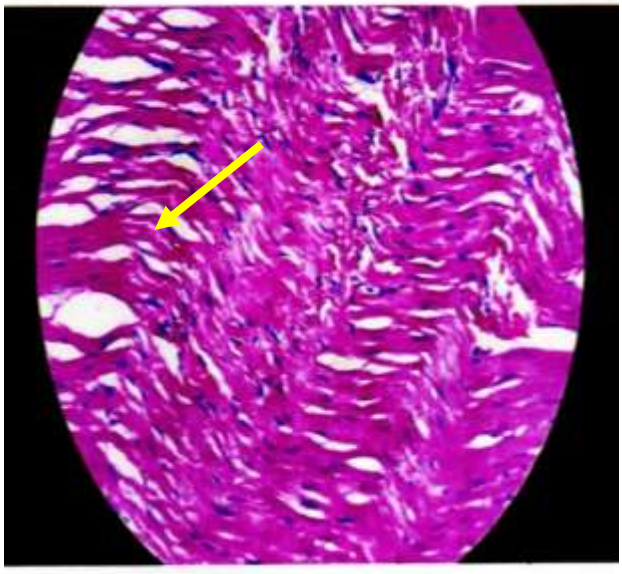


Figure 05: Histological features of the heart of group treated with *Nigella sativa* 500mg + Doxorubicin showing derangement of muscle fibers and hemorrhage (arrow mark)

Figure 06: Histological features of the heart of group treated with Carvedilol + Doxorubicin showing parallel arrangement of muscle fibers with peripherally located nuclei (arrow mark)

Discussion

Imbalance between oxidation and antioxidant in cellular environment causes cell damage ,and this imbalance has potential role in development of myocardial infarction leading to heart failure(13).Doxorubicin is an anticancer drug but nowadays its use has been reduced significantly because of its fatal cardiotoxicity and cardiomyopathy(14) During administration of DOX high levels of free radicals are formed which then produce oxidative myocardial damage(15). The possible mechanism proposed for cardiotoxicity by DOX include free radical induced myocardial injury i.e. increased oxidative stress and released of free radicals including superoxide anion (16). since cardiac tissue rich in mitochondria and it's function mostly depends on oxidative metabolism thus producing significant amounts of free radicals. Accumulation of redox active DOX in these organelles would enhance mitochondrial production of reactive oxygen species (ROS) and reactive nitrogen species (17). High cardiac tissue level of Doxorubicinol a metabolite of the conventional doxorubicin are associated with both functional and morphological changes consistent with the

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DOX induced cardiomyocyte injury(18) however, conventional doxorubicin induced myocardial damage not exactly known but is believed to involve production of free radicals species that induce peroxidation of myocytes membranes and subsequent influx of intracellular calcium(19**)**.however early morphological changes like cytoplasmic vacuolization and myofibrillar loss of myocytes are believed to be caused by dilatation of sarcoplasmic reticulum, but in more advance cases, such cellular changes along with cardiac remodeling eventually leads to left ventricular failure with increased mortality (20).Results of present study indicate that intraperitoneal administration of DOX in dose of 20 mg/kg significantly elevated the serum level of CK-MB, LDH, SGOT, SGPT indicating myocardial damage similar to other studies done (21,22) and this rise of serum enzymes were found to be statistically significant in the present study as well. Carvedilol which is nonselective beta blocker was used as a reliable cardioprotective agent by previous researchers (23). In the present study also, carvedilol served as standard cardioprotective drug.

It was interesting to note that CK-MB levels were lower in rats administered with *Nigella sativa*, and it is found to be more cardioprotective in doses of 500mg/kg than 250mg/kg. *Nigella sativa* causes significant reduction in LDH, SGOT, and SGPT levels also indicated by Ravichandra et al. (24). SGOT, SGPT are nonspecific indicators of cardiac injury but are known quantitative indices of compromised myocardial cells integrity caused by Doxorubicin. most medicinal plants exhibit therapeutic action because of presence of antioxidants and phytochemicals Thymoquinone, (25) and AENS demonstrate antioxidant property through different mechanisms in several recent reports. Thymoquinone acts as a free radical and superoxide radical scavenger and it preserve the activity of various antioxidant enzymes such as catalase, glutathione peroxidase and glutathione - S-transferase (26)

Myocardium of normal saline treated rats illustrated normal integrity of myocardial cell membrane and absence of any inflammatory cell infiltration. Doxorubicin injected rats showed separation of cardiac muscles fibers and inflammatory cell infiltrate, the reduced inflammatory cell infiltration and normal cardiac muscles fibers architecture in *Nigella sativa* treated rats further confirmed cardioprotective effects.

Although in the present study use of AENS have shown to possess cardioprotective potential, but further studies of longer duration are needed to explore the therapeutic efficacy of *Nigella sativa* as cardioprotective drugs.

Conclusion

In the present study it was found that administration of AENS significantly reduced the doxorubicin induced damage of rat myocardium without any harmful effects. This cardioprotective potential of *Nigella sativa* might be attributed to its antioxidant properties and the presence of chemical compounds like Thymoquinone, Secoisolariciresinol(SDG),Lignin ,soluble fibers present. this study also carries scope for further assessment of *Nigella sativa* with its hydroalcoholic extract, other dose levels and with extended test durations , other biochemical parameters ,isolation and structure determination of the cardio protective substances and a detailed explanation of the mechanism of action. Prolong study using large number of animals is required so that substantial data can be generated for facilitating further evaluation of this agent. based on

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current review it has been concluded that *Nigella sativa* has broad spectrum of therapeutic potential in cardiovascular diseases and further long-term human trials are required to established therapeutic utility.

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Conflict of interest—None declared

Ethical approval- This study was approved by Institutional Ethics Committee (Approval number-IAEC/2019/05 dated 20/07/2019

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