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Full Length Research Paper

Exploration of signal transduction mechanism of therapeutic action of *Solanum nigrum*

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In modern world, the recognition, understanding and treatment with phytomedicines have been increased during the last few decades. However, in spite of the progress in the field of research, the mechanism of therapeutic action of most of the phytomedicine remains unknown. Because of special interest with reference to our previous work, further in depth study was carried out on *Solanum nigrum* leaves and fruit (red and black varieties). The crude extracts of *S. nigrum* (black fruit has highest activity) was selected along with ethyl acetate, chloroform, *n*-butanol and aqueous fraction of both varieties leaves and seeds. The effect was observed with acetylcholine, atropine adrenaline and histamine in 1×10^{-2}, 1×10^{-4} and 1×10^{-6} molar concentrations. Remarkable observed results showed the involvement of muscarinic receptors to produce the therapeutic effects and it could be assumed that the present finding support the effect like acetylcholine through muscarinic receptors in *S. nigrum*. Acetylcholine is the neurotransmitter present at neurotransmitter junction, brain and gastrointestinal tract etc., therefore, the drugs having acetylcholine like effects have vital role in many diseases as the parasympathomimetic drugs, thus the discovery of new drugs with minimum side effects provides a great benefit to mankind.

Key words: *Solanum nigrum*, muscarinic receptor, acetylcholine, smooth muscles.

INTRODUCTION

The field of signal transduction mechanism with respect to herbal medicines has expanded tremendously thus opening new avenues of biological sciences. Understanding of signal transduction mechanism has relied upon the development of new and existing methods for herbal medicines. It is highly interesting to investigate different techniques for the exploration of mode of action of herbal medicines.

Different varieties of *Solanum nigrum* are found in other parts of the world including South Africa, Germany and America. It is used by European as a remedy for convulsions. In Southern Rhodesia, the plant is an African remedy for malaria, black water fever, dysenteries and other diseases. In Mauritius, a poultice of the plant is applied for the relief of abdominal pain and inflammation of the urinary bladder. It has also been used in the treatment of headaches, ulcers and wounds. Reported constituents are solanine, saponin, solamargine, solanigrine and solasodine, steroidal glycosides oil (Devi and Jainu, 2006; Harikrishnan et al., 2011), dihydroxy stearic, linoleic and oleic acid, tetrahydroxy stearic, linoleic and oleic acid (Chopra et al., 1958; Duke, 1985; Nadkarni, 1980).

For this purpose, successful investigations were carried out on isolated smooth muscle of rabbit intestine with crude extracts of two varieties of *S. nigrum* leaves and fruits (Mehjabeen et al., 2004). Further elaborative study have been conducted with *S. nigrum* leaves (black fruit variety), black fruit extract and fractions.

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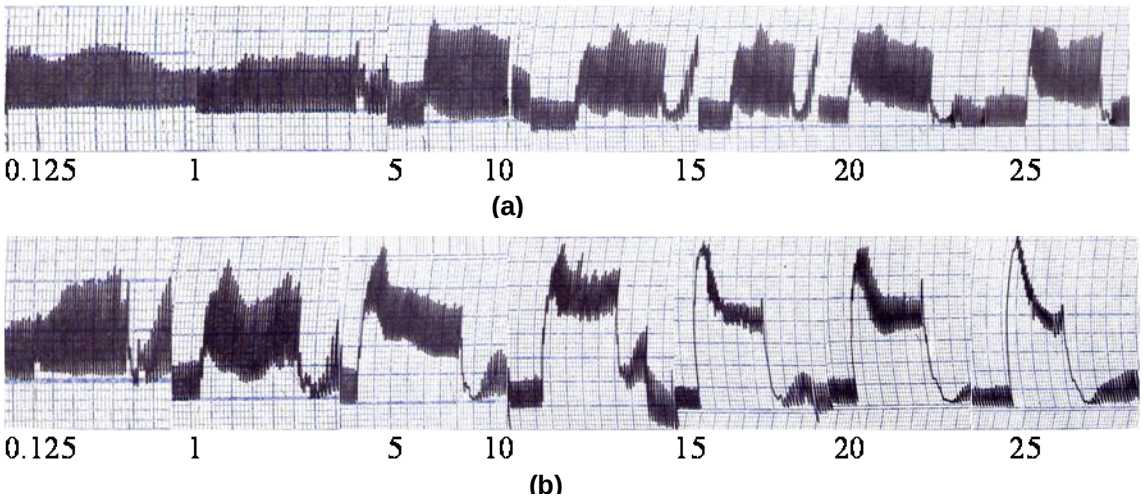


Figure 1. (a) Dose dependant effect of *S. nigrum* leave extract; SNBL (Black fruit variety); (b) Dose dependant effect of *S. nigrum* black fruit extract; SNBS (Black fruit variety).

MATERIALS AND METHODS

The leaves and fruits of *S. nigrum* (*S. nigrum* black fruit) were collected during January 2003 to 2005 (Voucher number 001116-04) from University of Karachi, Pakistan. The plants were identified by Professor Dr Mansoor Ahmad, Research Institute of Pharmaceutical Sciences, Karachi Unversity. The fresh leaves and fruits were reduced in size and macerated with ethanol for 15 days separately in a closed amber glass occasionally shaken at different intervals. The crude extracts were filtered, vacuum dried through rotary evaporator and stored at room temperature (25°C) until further uses. The ethyl acetate, chloroform, *n*-butanol and aqueous fractions of the crude extracts were fractionated by classical extraction methods (Ahmad et al., 2008).

Animals

Animals used in this study are adult rabbits (1.5 to 2.0 kg body weight) of either sex and local breed were housed at the Animal House of Research Institute of Pharmaceutical Sciences, University of Karachi, Pakistan, maintained at 23 to 25°C and were given standard diet and tap water *ad libitum*. Experiments were performed according to the method described by Mehjabeen et al. (2004), and Shah et al. (2010). The animals handling were carried out according to the rules and regulation of ethical committee of Research institute of Pharmaceutical Sciences and prior approval was obtained.

Methods

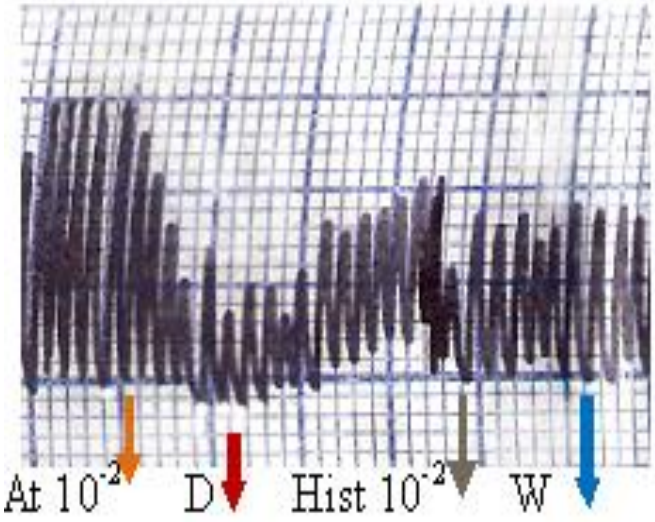
Animals were sacrificed by a blow on the back of the head; the abdomen was cut open and a piece of jejunum was taken out. Segments of 2 cm long were suspended in Tyrode's solution aerated with a mixture of 95% oxygen and 5% carbon dioxide, and maintained at 37°C. The spontaneous intestinal movements were recorded isotonically using Harvard transducers and Harvard Student Oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these experimental conditions, the rabbit jejunum exhibits spontaneous rhythmic contractions and therefore allows study of the relaxant (spasmolytic) activity directly without the use of an agonist. To determine the effects of plant extract on spontaneous movements of intestine, crude extract and their fractions were dissolved in 1 ml of distilled water and thereafter, it was added to the organ bath after equilibration period. The effects of crude extract and their fractions on the contraction and relaxation pattern of isolated rabbit intestine (smooth muscles) were recorded (Mehjabeen et al., 2004; Jahan et al., 2004; Blattner et al., 1978).

Chemicals

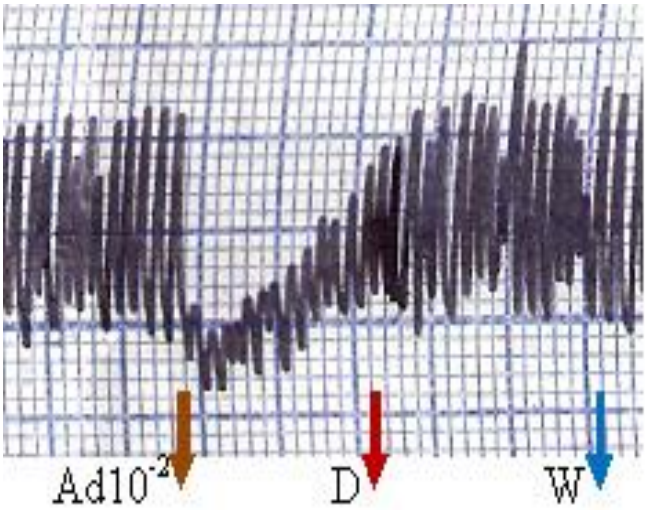
Reference drugs acetylcholine perchlorate, adrenaline, histamine and atropine were purchased from Sigma Chemicals Co., St. Louis, MO, USA while Verapamil was obtained from Abbott Laboratories. All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh on the day of the experiment. The vehicle used for solubilization of drugs had no effect on tissue contractility in the control experiments.

RESULTS

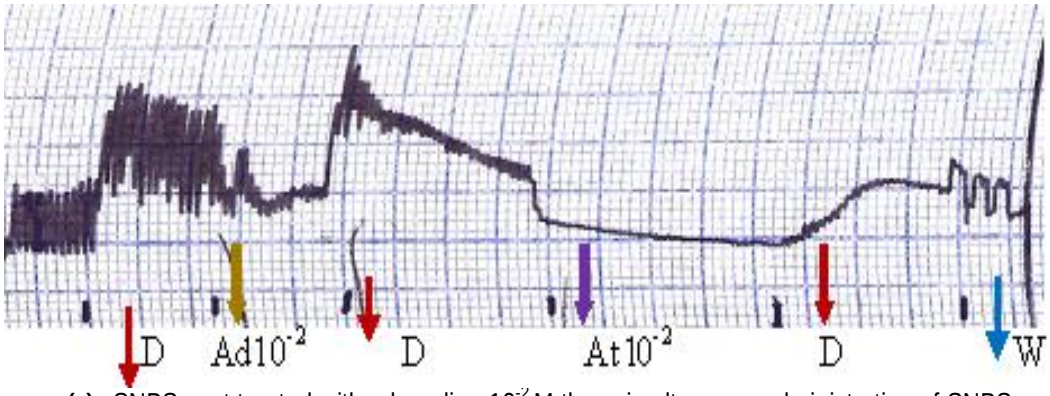
The pharmacological activity (*in vivo* on isolated rabbit intestine) of the crude extracts of SNBL (*S. nigrum* black variety leaves) and SNBS (*S. nigrum* black variety fruits) crude extract were observed at the doses of 0.125, 1, 5, 10, 15, 20 and 25 mg (Figure 1). The effect of the test drug response was observed through the contraction and relaxation of intestine of rabbits. Both of the extracts showed dose dependant spasmogenic effect. Figure 2a to k and Tables 1 to 3 presented the agonist, antagonist and synergistic response of the crude extract SNBS with standard drugs (adrenaline 1×10^{-2}, Histamine 1×10^{-2}, verapamil 1×10^{-2}, neostigmine 5×10^{-2}, atropine 1×10^{-2} and acetylcholine 1×10^{-2}). To determine the possible mechanism of action, the crude extract of SNBS was tested at the dose of 15 mg/ml.



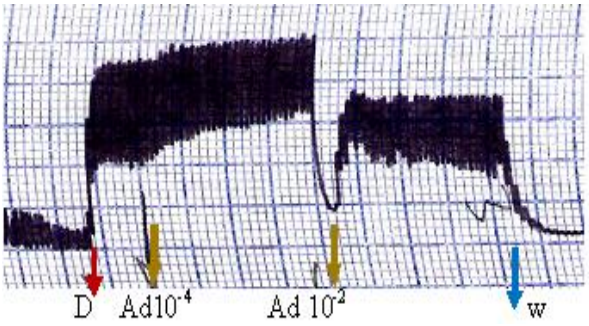
(a) SNBS with pretreated atropine, 10^{-2} and post treated histamine, 10^{-2} M.



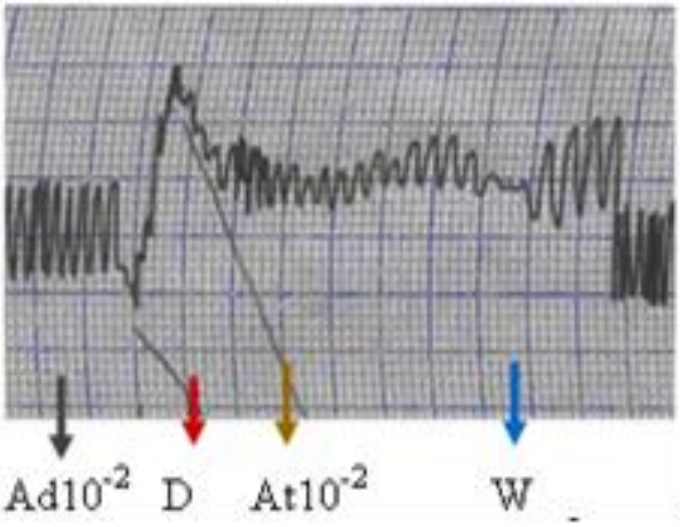
(b) SNBS with pretreated adrenaline, 10^{-2}M



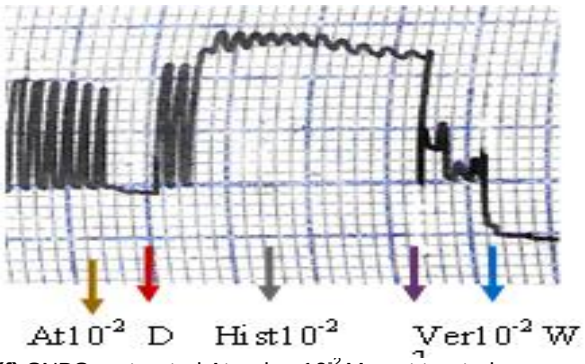
(c) SNBS post treated with adrenaline 10^{-2} M than simultaneous administration of SNBS, atropine 10^{-2} and SNBS.



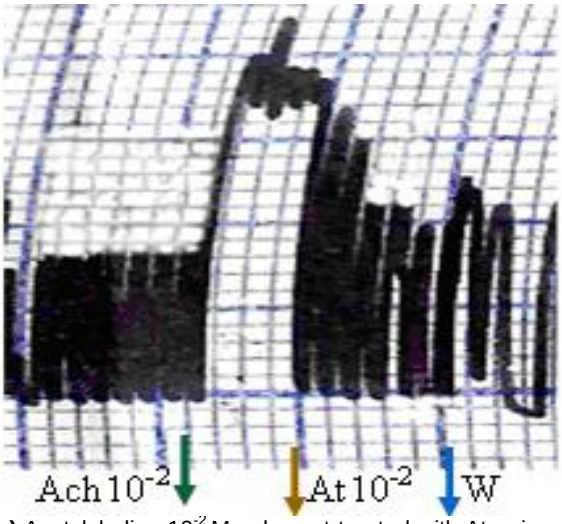
(d) SNBS with post treated adrenaline 10^{-4} and 10^{-2} M.



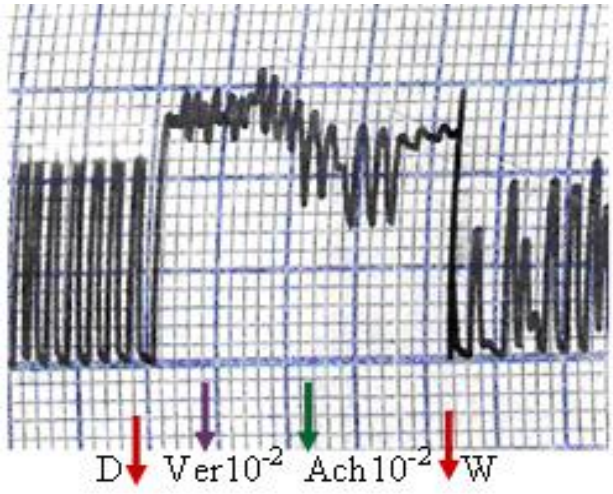
(e) SNBS with pretreated Adrenaline10^{-2} M and Atropine 10^{-2} M.



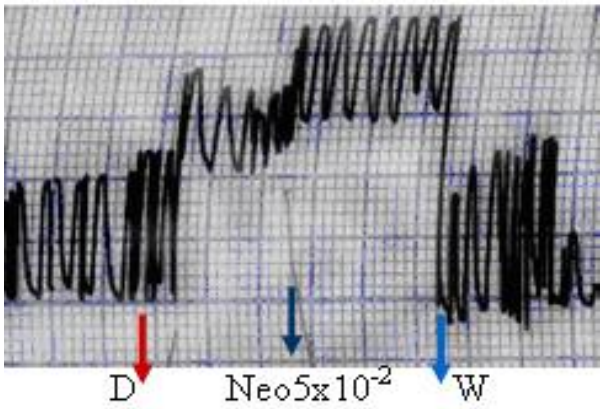
(f) SNBS pretreated Atropine 10^{-2} M post treated Histamine 10^{-2} M and Verapamil 10^{-2} M



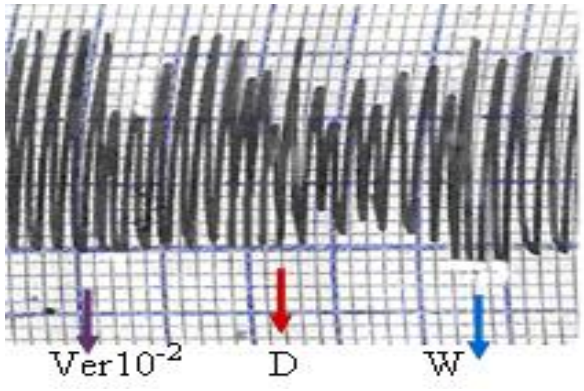
(g) Acetylcholine 10^{-2} M only post-treated with Atropine 10^{-2} M.



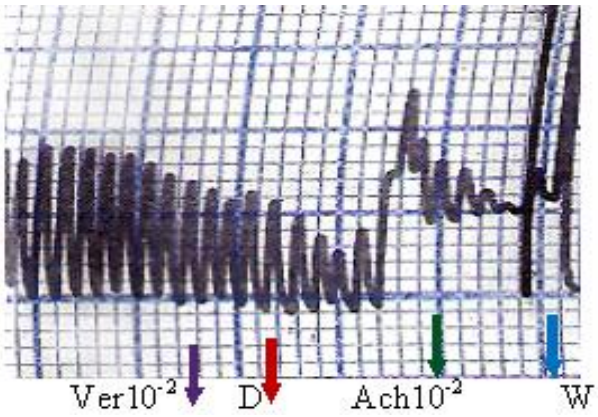
(h) SNBS post treated with verapamil10^{-2} M and Acetylcholine 10^{-2} M.



(i) SNBS post treated with Neostigmine 5×10^{-2} M.



(j) SNBS pretreated with Verapamil 10^{-2} M.



(K) SNBS pretreated with Verapamil 10^{-2} M and post treated Acetylcholine 10^{-2} M.

Figure 2a-k. The effect of crude extract of *S. nigrum* (SNBS) *on* the isolated rabbit intestine with standard. D = dose of crude extract; W = wash; SNBS = *S. nigrum* black berries.

Table 1. Comparative effect of crude extract of SNBS (dose 15 mg/ml) with standard drugs (Mean±SEM).

Treatment 1	Response	Treatment 2	Response	Treatment 3	Response	Treatment 4	Response
Control	1±0.02	Control	1.5±0.05	Control	1.5±0.07	Control	0.5±0.04
Adrenaline 1×10^{-2}	0.1±0.03	Atropine 1×10^{-2}	0.5±0.04	Adrenaline 1×10^{-2}	0.1±0.01	SNBS	1.4±0.06
SNBS	1.3±0.02	SNBS	0.4±0.03	SNBS	1±0.05	Neostigmine 5×10^{-2}	2.3±0.08
Histamine 1×10^{-2}	1.5±0.03	Histamine 1×10^{-2}	1±0.03	Wash	0.9±0.04	Wash	0.5±0.05
Verapamil 1×10^{-2}	0.5±0.03	Wash	0.4±0.02				
Wash	0.3±0.01						

Table 2. Comparative effect of crude extract of SNBS (dose 15 mg/ml) with standard drugs (Mean±SEM).

Treatment 5	Response	Treatment 6	Response	Treatment 7	Response	Treatment 8	Response
Control	0.5±0.04	Control	1±0.04	Control	0.9±0.05	Control	1±0.03
SNBS	1.7±0.06	SNBS	1.5±0.06	Verapamil 1×10^{-2}	0.4±0.05	SNBS	1.6±0.05
Adrenaline 1×10^{-2}	0.6±0.03	Verapamil 1×10^{-2}	1.2±0.05	SNBS	0.6±0.01	Atropine 1×10^{-2}	1±0.03
SNBS	2±0.08	Acetylcholine 1×10^{-2}	1.1±0.04	Acetylcholine 1×10^{-2}	0.8±0.03	Wash	0.8±0.02
Atropine 1×10^{-2}	0.1±0.02	Wash	0.6±0.04	Wash	0.5±0.04		
SNBS	0.4±0.02						
Wash	0.3±0.01						

Table 3. Comparative effect of crude extract of SNBS (dose 15 mg/ml) with standard drugs (Mean±SEM).

Treatment 6	Response	Treatment 7	Response	Treatment 8	Response
Control	1±0.03	Control	1±0.06	Control	1±0.03
Verapamil 1×10^{-2}	0.4±0.03	SNBS	1.6±0.07	Verapamil 1×10^{-2}	0.4±0.03
SNBS	0.5±0.04	Neostigmine 5×10^{-2}	1±0.04	SNBS	0.6±0.04
Wash	0.4±0.02	Acetylcholine 1×10^{-2}	0.8±0.04	Wash	0.8±0.03
		Wash	0.6±0.04		

The crude extract SNBL and SNBS showed maximum activity at 20 and 25 mg/ml. The crude extract SNBS at 15 mg/ml was chosen to carry out effect of a drug with standard drugs because of more potent effect. The tracing of crude extract SNBS with different standard drugs were represented in Figure 2 and graphically in Figure 3.

DISCUSSION

The effect of the two varieties of *S. nigrum* crude extract and its ethyl acetate, chloroform, *n*-butanol and aqueous fractions along standard drugs were observed through the contraction and relaxation of isolated intestine of rabbits (Mehjabeen et al., 2004). In this study, crude extracts of *S. nigrum* (black fruit variety) were divided into two parts;

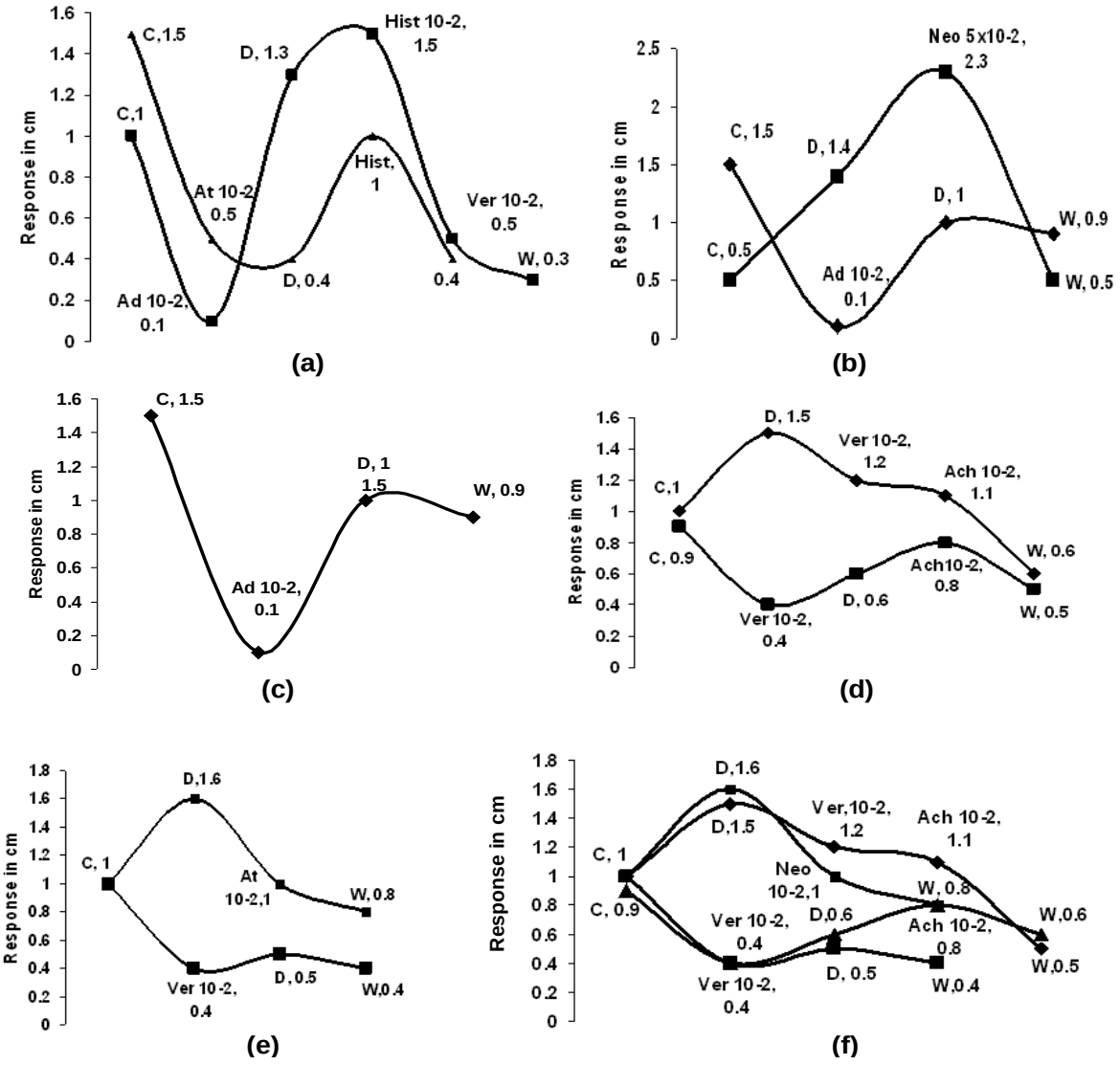


Figure 3a-f. Effects of crude extract of *S. nigrum* (SNBS) with standard drugs.

extract SNBL (leaves) and SNBS (fruits) and pharmacological investigation were performed *in vitro* on isolated rabbit intestine.

Tracings represent the dose related response of crude extract of *S. nigrum* on isolated rabbit intestine (Figure 1). The prominent smooth muscle contraction activity of the crude extract was recorded at a dose of 5 mg/ml. It was also observed that the response of the crude extract gradually increased as the dose increased. The overall response of the extracts on isolated tissue of rabbit intestine was increasing in the tone of smooth muscle which was maximum at the dose of 25 mg/ml.

All extracts whether crude or fractions showed parasympathomimetic like acetylcholine (Mehjabeen et al., 2004). This remarkable activity developed an interest of further investigation on mechanism of action. When tissue was first treated with atropine that stopped the intestinal activity, extract SNBS was then introduced and it produced its contraction of the intestinal muscle although muscarinic receptors were blocked by atropine (Wallis and Napier, 1999), simultaneously histamine was administered and the tissue still produced contractile effect. But upon addition of verapamil (calcium blocker), the effect was decreased. On the other hand, tissue was pretreated with verapamil and then extract SNBS was added; the effect of drug was not produced whereas pretreatment of tissue with adrenaline did not alter the response of the drug on tissue. The tissue that was pretreated with atropine did not allow the producing effect of the drug, but histamine had an effect on it. When the activity of the tissue was blocked by verapamil, the drug was then introduced but did have an effect on the tissue, and even the simultaneous administration of acetylcholine did not produce its classical effect. These

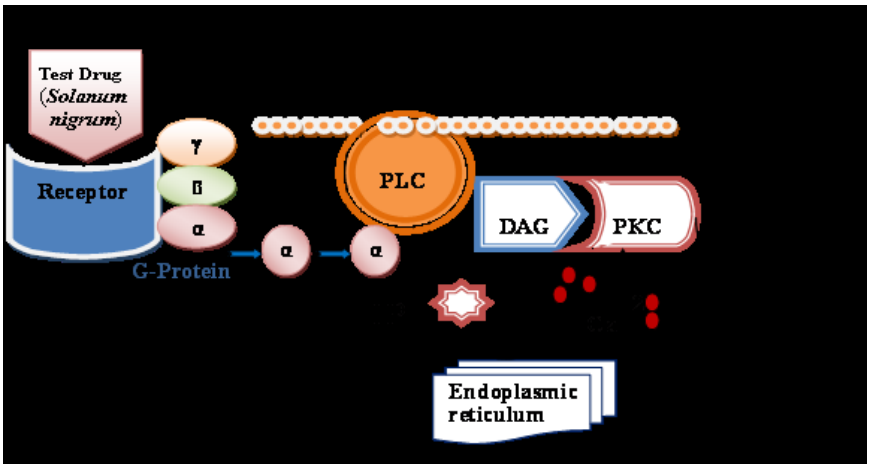


Figure 4. Mechanism of action and receptor involvement in test drug (*S. nigrum*).

findings indicated that there is an involvement of muscarinic receptor and influx of calcium to produce profound contractions. In our previous findings, both extracts SNBL and SNBS were fractionated and treated with standard drugs. Aqueous fractions pre-treated and post-treated tissue with atropine did not allow the production of muscarinic response. The effect of aqueous fraction of drug was transiently reduced because of compensatory mechanism, after the treatment of tissue with adrenaline than when it was increased. The effect of *n*-butanol fraction of crude ex-tract B post-treated with atropine and pre-treatment with adrenaline showed that adrenergic receptor blockage did not alter the response of the drug but atropine reduced it. In acetylcholine fraction, same results were observed. Overall, it is clear that crude extract SNBS and the fraction of both extracts showed the involvement of M_1 and M_3 receptors (Caulfield and Birdsall, 1998) at intestinal level.

The present findings showed that smooth muscle of rabbit intestine (ileum and jejunum) produce contraction with test drug like acetylcholine and at different doses even more potent effects were produced. This indicated the involvement of cholinergic receptor and possibility of three mechanisms responsible for the contraction by *S. nigrum*: (a) Activation of muscarinic receptor; (b) Stimulation of acetylcholine release from cholinergic nerve ending, and (c) Inhibition of acetylcholine esterase enzyme which causes the degradation of acetylcholine.

With the reported findings by Mishra and Raviprakash (1980) and Sawyer and Ehlert (1998), neostigmine 5×10^{-2} M /ml was added which did not alter response of tissue. Moreover the contraction produced by extract SNBL and SNBS was not reduced in cooled preparation (Ambach, 1946). This gave an idea that contractile response of extract SNBL and SNBS was non neurogenic response. To observe the effect of extract SNBL and SNBS by an inhibition of Ach E; the method used was described by Kela et al. (1995). For this purpose, extract SNBL and SNBS were added simultaneously with neostigmine; there was no potentiated response but when acetylcholine and neostigmine were added, there was an increased response. Similarly, extract A and acetyl-choline gave an additional contraction. Acetylcholine is the major neurotransmitter in the gastrointestinal region and initiates smooth muscle contraction by interacting with G-protein coupled muscarinic receptor (Figure 4) (Bolton, 1979; Hejazian–Y et al., 2011).

Conclusion

On the basis of results of the study, our attention is directed towards direct involvement of muscarinic receptor. It was also observed that when extract SNBL and SNBS produced contraction even after 5 or 10 min continuous in contact with receptor after washing, the tissue attained its normal position. That also gave an idea about the involvement of muscarinic receptor.

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