Pakistan J. Zool., vol. 51(1), pp 395-398, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.1.SC11

Short Communication

Study of Antiviral Potential of Cholistani Plants against New Castle Disease Virus

Mirza Imran Shahzad[1], Hina Ashraf[2,*], Muhammad Arshad[3], Sabeeha Parveen[4], Amna Aslam[4], Nargis Naz[4], Zahid Kamran[1], Sumbul Gohar Khalid[5], Sajid Hameed[1], Muhammad Ashfaq[6] and Muhammad Mukhtar[7]

[1]*University College of Veterinary and Animal Sciences, The Islamia University, Bahwalpur*
[2]*Department of Botany, Govt. Sadiq College Women University, Bahawalpur*
[3]*Department of Basic Sciences, College of Veterinary and Animal Sciences, Jhang*
[4]*Department of Botany, The Islamia University, Bahawalpur*
[5]*Department of Bioinformatics and Biotechnology, Islamic International University Islamabad*
[6]*Department of Chemistry, The Islamia University of Bahawalpur*
[7]*Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Malaysia*

Article Information
Received 28 September 2017
Revised 12 January 2018
Accepted 28 March 2018
Available online 11 January 2019

Authors' Contributions
MIS, NN and MM supervised the work. HA, SP and AA conducted the research. MIS designed. MA, SKG and SH facilitated in development of antiviral assays. ZK and MA provided administrative and financial support to this research.

Key words
NDV, Antiviral activity, Cholistan, *In Ovo* assay.

ABSTRACT

Present study is based on evaluation of antiviral potential of methanolic extracts of eleven selected Cholistani plants against New Castle Disease Virus (NDV)-LaSota strain. All of these plants were reported for their different pharmacological activities but their antiviral potentials were not known before. The methanolic extracts were made and concentrated by rotary evaporator and finally dissolved in distilled water before taking their antiviral trials in 7-11 days old chicken embryonated eggs. The viral loads were determined through heamagglutination (HA) test. The methanolic extract of each plant was found effective against NDV but in varying order. The active extracts were further used in different concentrations and IC_{50} of each extract was calculated. The extract of *Achyranthes aspera* was found most effective with IC_{50} 3.125µg and the extract of *Oxystelma esculentum*, was found least effective with IC_{50} 60µg in the series. The current study has established the fact that Cholistani plants are rich source of anti-NDV agents and can be used in purified or in crude form.

Exploiting plants for medicinal purposes is the biggest example of human's utilization of nature. Although market is now flooded with no. of antimicrobial agents but demand of new drugs especially antiviral drugs is continuously rising. Extracts, powders, herbal teas, oil based emulsions of plants are already in use to treat viral diseases from hundreds of years but demand for new compounds is increasing (Vijanyan *et al*., 2004). Higher plants are rich source of antiviral drugs (Jassim and Naji 2003; Aslam *et al*., 2016; Shahzad *et al*., 2017). Recently a compound Nordihydroguaiaretic acid (NDGA) was isolated from leaves of Larrea divaricate and found very effective against Junin virus (Konigheim *et al*., 2005). Similar, compound was isolated from other species of genus Larrea and successfully tested against Herpes Simplex Virus (HSV) type I and II. These NDGA compounds are lignin in nature and they have inhibitory effect on transcription process of HIV viruses (Gnabre *et al*., 1995). Other plant based principal antiviral agents include proteins, polypeptides, peptides, furyl, sulphides, flavonoids, poly phenolics, terpenoids, coumarins and saponins (Cowan 1999). These plant based compounds are not only safe to use, easy and cheap to produce but also found more effective as compared to synthetic compounds. The studies have proved that plant based products work in more synergistic way and provide less chance to microbes to develop resistance against them (Solanki, 2010).

Newcastle disease (ND) or commonly called as Rani Kheit is a highly infectious viral disease which affects poultry of all ages (Shahzad *et al*., 2011). ND outbreaks were on continuous rise during the period of 2011- 2012 in country. Most of the young commercial broiler flocks were damaged. There is no known treatment of ND in market except extensive use of vaccine (Kitazato *et al*., 2007). By keeping in view the devastating effects of ND on poultry industry and natural resourcefulness of Cholistani plants, the current study was designed to evaluate their antiviral potentials against NDV and provide cost effective remedy from local resources.

* Corresponding author: hinaimranmirza@gmail.com
0030-9923/2019/0001-0395 $ 9.00/0

Materials and methods

Eleven fresh plants including Achyranthes aspera,Haloxylon recurvum,Haloxylon salicornicum, Oxystelma esculentum,Octhocloa compressa,Neurada procumbens, Panicum antidotale, Salsola baryosma, Suaeda fruticosa, Sporobolus icolados and Solanum surattense were collected from herbarium and surroundings of Cholistan Institute of Desert Studies, Baghdad ul Jadeed Campus, The Islamia University of Bahawalpur. The plants were identified and authenticated by taxonomists at Department of Botany, The Islamia University of Bahawalpur and their vouchers were saved. New Castle disease virus (NDV) was obtained from depository of Biochemistry and Molecular Biology Lab, University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur and specific pathogen free (SPS) 9-11 days chicken embryonated (CE) eggs were obtained from Govt Hatchery, Model town A, Bahawalpur. The NDV was propagated in 9-11 days old CE eggs via chorioallantoic route (Sulaiman *et al*., 2011).

For preparation of methanol extracts, the whole plants were collected and dried under shade at room temperature (RT) for 10 days. The dried plants were grinded by lectric grinder and kept in airtight containers at RT. Methanol extracts were made by dissolving 10 g powder of each plant in 200 mL methanol. The suspensions were kept in airtight containers for 96 h at constant shaking. Later, the samples were subjected to rotary evaporator and concentrates were rinsed with methanol and chloroform mixture and allow them to re-evaporate. Finally the precipitates were weighed and dissolved in 10 mL distilled water, vortexed, filter sterilized and stored at -20 °C (Joshi and Kaur, 2013.

For viral inoculations, 9-11 days old CE eggs were candled before inoculations. The broader ends of eggs were swabbed with 70% alcohol and drilled manually with sterile needle. The inoculum was injected and hole was sealed with molten wax. The inoculated eggs were incubated at 37°C for 48 h. Later the eggs were harvested and HA test was performed (Wang *et al*., 2008).

Standard HA test was optimized to quantify the titer of ND virus.

For *in ovo* antiviral assay, the filter sterilized plant extracts were mixed with equal vol. of viral inoculums and injected in 9-11 days old CE eggs. Different concentrations of plant extracts (25-100 mg) were tested against NDV. Autoclave distilled water was used as negative control and NDV as virus control. HA titers were determined after each experiment and change in HA titer as compared to virus control was noted (Table I). IC_{50} of each extract was calculated by serial dilution method.

Results and Discussion

The desired HA titer *i.e.* 2048 of NDV was achieved after 6th passage.

In initial experiments, when 100 mg dose of plant extracts were tested against NDV eight out of eleven extracts were found very effective and kept HA titers at 0. The remaining three plant extracts were also effective but their HA titers were 8 (Table I). In later experiments, different concentrations of plant extracts were were made and tested against NDV and IC_{50} value of each extract was calculated. The methanolic extracts of *A. aspera* and *H. salicornicum* were best among all and their IC_{50} values were 3.125 and 6.25 mg, respectively. Among other plants the extracts of *S. surattense*, *S. fruticosa O. compressa* and *H. recurvum* have shown 100% anti-NDV activity in their higher concentrations including 100 and 75, respectively but these plants lost the potential gradually in lower concentrations (Table I). The extracts of plants *N. procumbens* and *O. esculentum* were next in order and they have controlled NDV very effectively only in higher concentrations (100 mg) but later they lost their potential gradually. The potency of these extracts has direct relationship with concentration of extracts and their antiviral activities. The antiviral potentials of plant extracts of *P. antidotale*, *S. baryosma*and *S. icolados*, were least effective in antiviral category and none of them has reduced HA titer at 0 through any concentration (Table I).

All plant extracts were found effective against NDV but in varying order. Initially, all plant extracts were tested at higher concentrations *i.e.* 100 mg all of them were found effective against NDV. It was worth mentioning that all plant extracts were found nontoxic at 100 mg and all embryos were found alive after inochoulations and similarly, no change in shape of RBCs, rupturing of blood vessels and hemolysis was seen by these extracts. The extracts of *A. aspera* and *H. Salicornicum* plants were the most potent and rich source of anti-NDV agents. These extracts were equally effective at 25 mg concentration as they were effective at 100 mg concentrations. The extracts of rest of plants were also found effective but in varying order. Even the lowest ranked extract of *S. baryosma*, was active against NDV at 100 mg concentration. These results indicate that Cholistani plants are naturally rich source of anti-NDV agents. There is no known drug against NDV except few herbal products (Mabiki *et al*., 2013). Similar studies were done by Bedoya *et al*. (2001) on plants from Iberian Peninsula and screened various medicinal plants of this region for their anti HIV activity on HIV-infected cells. Wang and Lu (1995) has reported antiviral activity from extracts of *Geranium carolinianum* L. In another report, its aqueous extract was found effective against HBV and help in improving the clinical data of patients (Zhu and

Ren, 1995). In recent studies done by Gescher *et al*. (2011) the aqueous extract of *Rhododendron ferrugineum* was positive against Herpese Simplex Virus type 1 (HSV-1) while negative against adenovirus.

Table I.- Anti-NDV activities from methanolic extracts of Cholistani plants and their IC_{50}.

Medicinal plant	Concent. of extract (mg/0.1 mL H_2O)	HA titer after challenge	IC_{50} (mg/0.1 mL H_2O)	Control virus
S. surattense	25	8	6.25	1024
	50	4		2048
	75	0		1024
	100	0		2048
A. aspera	25	4	3.125	2048
	50	0		1024
	75	0		2048
	100	0		2048
S. baryosma	25	512	50	1024
	50	128		2048
	75	32		2048
	100	8		1024
O. esculentum	25	512	60	2048
	50	256		2048
	75	64		1024
	100	0		2048
P. antidotale	25	32	12.5	1024
	50	32		2048
	75	16		2048
	100	8		2048
S. icolados	25	32	12.5	1024
	50	32		1024
	75	8		2048
	100	8		4096
O. compressa	25	32	6.25	1024
	50	08		2048
	75	0		1024
	100	0		2048
S. fruticosa	25	32	12.5	1024
	50	4		2048
	75	0		1024
	100	0		2048
H. recurvum	25	256	35	2048
	50	32		2048
	75	0		2048
	100	0		2048
H. salicornicum	25	32	6.25	2048
	50	0		2048
	75	0		2048
	100	0		2048
N. procumbens	25	128	25	2048
	50	64		2048
	75	4		2048
	100	0		2048

The antiviral research study against AIV by Ehrhardt *et al*. (2007) provides the basis for further research on plant based anti-adhesive compounds. The results of these studies were in accordance of results of other researchers like Gonzalez *et al*. (2012). They have reported a plant based compound Fucoidan from *C. Okamuranus*, which was found very active against NDV. Similarly antiviral activities against Vaccinia, NDV and HBV were reported by Premnathan *et al*. (1992) by using methanol extracts of mangroves, seagrasses and seaweeds, of India's southeast coastal region. Similarly, Usha and Sharma (2012) have tested aqueous, ethanol and methanol extracts of *C. crista* L. and found them effective against NDV. Similar results were found from fruit pulp and leaf extracts of *Momordica balsamina* against NDV (Chollom *et al*., 2012). Similar studies against other poultry viruses are also reported like Ahmed *et al*. (2014) has reported anti-IBDV activity from ethanol extracts of *Glycyrrhiza glabra*, *Moringa oleifera*, *Phyllanthus emblicus* and *Eugenia jambolana*. Pant *et al*. (2012) have proved that hydro- alcoholic extract of *Withania somnifera* roots was rich in anti-IBDV compounds. Similarly, Park (2003) has reported anti-AIV activity from methanol extracts of four different Korean plants. Ramzi *et al*. (2006) have reported antiviral activities against AIV and HSV-1 from their methanol and hot aqueous extracts of *Boswellia ameero, Boswellia elongata*, *Buxus. hildebrandtii*, *Cissus hamaderohensis*, *Dracaena cinnabari*, *Exacum affine*, *Cleome socotrana*, *Jatropha unicostata* and *Kalanchoe farinacea*. Chen *et al*. (2014) have studied moderate anti infectious bronchitis virus (IBV) activity from ethanol extracts of *Sambucus nigra* fruit. Different researchers have suggested different mechanisms of antiviral activities from different extracts of medicinal plants, some have anticipated the loss in attachment of the virus to host cell surface especially at early stages of infection or some have suggested the inhibition of essential enzymes required for growth or multiplication of virus. Some have reported the protein or polypeptide nature of antiviral compounds (Bajpai and Chandra, 1990).

Conclusions

There is no known treatment of New Castle Disease except some herbal extracts. Cholistani plants have highlighted huge antiviral potential to treat NDV. Although Cholistani plants were reported for number of pharmacological activities but this is the first report of their antiviral potential especially against NDV.

Acknowledgements

We acknowledge the services provided by Govt. Poultry Farm, Model Town A Bahawalpur in terms of

providing embryonated eggs. We are thankful to Mr. Ghulam Sarwar, Lecturer, Department of Life Sciences, who has helped in taxonomic characterization of plants. Similarly, we are thankful to Mr. Izhar, Lab Attendant, Biochemistry and Mol. Biology Lab, UCVAS at The Islamia University of Bahawalpur.

Statement of conflict of interest

The authors have no conflict of interest.

Refferences

Ahmed, W., Ejaz, S., Anwer, K. and Ashraf, M., 2014. *Cen. Eur. J. Biol.,* **5**: 531-542.

Aslam, A., Shahzad, M.I., Parveen, S., Ashraf, H., Naz, N., Zehra, S.S., Kamran, Z., Qayyum, A. and Mukhtar, M., 2016. *Pak. Vet. J.,* **36**: 302-306.

Bajpai, S.K. and Chandra, K., 1990. *Fitoterpia*, **61**: 3-8.

Bedoya, L.M., Palomino, S.S., Abad, M.J., Bermejo, P. and Alcami, J., 2001. *J. Ethnopharmacol.,* **77**: 113-116. https://doi.org/10.1016/S0378-8741(01)00265-3

Chen, C., David, M.Z., Brantley, S., Sharpe, M., Childress, K., Hoiczyk, E. and Pendleton, A.R., 2014. *BMC Vet. Res.,* **10**: 1746-6148. https://doi.org/10.1186/1746-6148-10-24

Chollom, S.C., Olawuyi, A.K., Danjuma, L.D., Nanbol, L.D., Makinde, I.O., Hashimu, G.A., Alesa, M.U., Esilonu, J.T., Ogundeji, E.B. and Kwatjel, J.S., 2012. *J. Adv. Pharm. Edu. Res.,* **3**: 82-92. https://doi.org/10.4103/2231-4040.97274

Cowan, M. M., 1999. *Clin. Microbiol. Rev.,* **12**: 564-582.

Ehrhardt, B., Hrincius, E.R., Korte, V., Mazur, I., Droebner, K. and Poetter, A., 2007. *Antivirol. Res.,* **76**: 38-47. https://doi.org/10.1016/j.antiviral.2007.05.002

Gescher, K., Kühn, J., Hafezi, W., Louis, A., Derksen, A., Deters, A., Lorentzen, E. and Hensel, A., 2011. *Fitoterapia,* **82**: 408-413. https://doi.org/10.1016/j.fitote.2010.11.022

Gnabre, J.N., Bates, R.B., Caldera, S. and Huang, R.C., 1995. *Tetrahedron,* **51**: 12203-12210.

Gonzalez, F.E., Suarez, E.C., Marie, D.E., Gamboa, E.M., Padilla, C.R. and Avila, L.M.T., 2012. *Virol. J.,* **9**: 307. https://doi.org/10.1186/1743-422X-9-307

Jassim, S.A.A. and Naji, M.A., 2003. *J. appl. Microbiol.,* **95**: 412-427. https://doi.org/10.1046/j.1365-2672.2003.02026.x

Joshi, M. and Kaur, S., 2013. *Asian J. Pharm. Clin. Res.,* **6**: 25-28.

Kitazato, K., Wang, Y., Kobayashi, N., 2007. *Theriogenology*, **1**:14-22.

Konigheim, B.S., Golenioswki, M.E. and Contigiani, M.S., 2005. *Drug Design Rev.*, **2**: 81-83.

Mabiki, F.P., Mdegela, R.H., Mosha, R.D. and Magadula, J.J., 2013. *J. Med. Pl. Res.,* **7**: 863-870.f

Pant, M., Ambwani, T. and Umapathi, V., 2012. *Ind. J. Sci. Technol.,* **5**: 2750-2751.

Park, K.J., 2003. *Phytother. Res.,* **17**: 1059-1063. https://doi.org/10.1002/ptr.1259

Premnathan, M., Chandra, K. and Bajpai, S.K., 1992. *Bot. Mar.,* **35**: 321-324. https://doi.org/10.1515/botm.1992.35.4.321

Ramzi, A., Mothana, A. and Mentel, R., 2006. *Phytother. Res.,* **20**: 298-302. https://doi.org/10.1002/ptr.1858

Shabbir, M.Z., Zohari, S., Yaqub, T., Nazir, J., Shabbir, M.A., Mukhtar, N., Shafee, M., Sajid, M., Anees, M., Abbas, M., Khan, T.M., Ali, A.A., 2013. *Virol. J.,* **10**: 170-173.

Shahzad, M.I., Iqbal, A., Ali, F., Sial, N., Ashfaq, M., Hasanat, A. and Khanum, A., 2017. *Pakistan J. Zool.*, **49**: 1057-1062. https://doi.org/10.17582/journal.pjz/2017.49.3.1057.1062

Shahzad, M., Rizvi, F., Khan, A., Siddique, M. and Khan, M.Z., 2011. *Int. J. Agric. Biol.,* **13**: 266-270.

Solanki, R., 2010. *Int. J. Comp. Pharmacol.,* **1**: 1-4.

Sulaiman, L.K., Oladele, O.A., Shittu, I.A., Emikpe, B.O., Oladokun, A.T. and Meseko, C.A., 2011. *Afr. J. Biotech.,* **20**: 4256-4258.

Usha, P. and Sharma, M.C., 2012. *Glob. J. Res. Med. Pl.,* **1**: 440-447.

Vijanyan, P., Raghan, C., Ashok, G., Dhanaraj, S.A. and Suresh, B., 2004. *J. Med. Res.,* **120**: 24-29.

Wang, J.X., Zhou, J.Y., Yang, Q.W., Chen, Y., Li, X., Piao, Y.A. and Li, H.Y., 2008. *J. Virol. Meth.,* **153**: 218-222. https://doi.org/10.1016/j.jviromet.2008.07.015

Wang, Y.L. and Lu, J.J., 1995. *J. Trad. Chinese Ophthalmol.,* **5**: 78-82.

Zhu, M. and Ren, J.J., 1995. *Acta Acad. Med. Suzou,* **15**: 11-22.