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RESEARCH ARTICLE

NEW TRENDS IN CANCER THERAPY AND ANTIVIRAL DRUG RESEARCH.

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Abstract

The search for new trends in cancer therapy and viral infection control has attracted the attention of many scientists nowadays. Egyptian snake, scorpion venoms and L-amino acid Oxidase (LAO) were investigated for their anticancer and antiviral potentials. Cytotoxic effect of test venoms to cancer cell lines was recorded with up to 80 and 63 fold increasein case of LAO enzyme and scorpion venom respectivelycompared to normal cell line. Venom induced apoptosis was evaluated using semi quantitative RT-PCR and revealed up-regulation of pro-apoptotic genes while down regulation of anti-apoptotic gene. A decrease in size of tumor mass and ascetic fluid volume was also observed in laboratory animals post venom treatment.Snake andscorpionvenom treated Vero cells showed a reduction in Herpes simplex virus-II(HSV) infectivity titer by 30% and 40% respectively as well as a marked decrease in Vesicular Stomatitis Virus (VSV) titer post venom treatment. It was apparent that the viricidal potentials exceeds the antiviral one, where VSV was completely inactivated by most venoms within 6 hrspost treatment, while HSV reduction titer was about 65% to 50%,6 hrs post treatment. Data suggested that theses venoms could present a new approach in anti-tumoral and anti-viral drug research

Introduction:-

Cancer is characterized by an accelerated and uncontrolled multiplication of a set of aberrant cells which lose their apoptotic ability. Research has been undertaken in order to find out the factors which promote uncontrolled multiplication of cells and how cancer genes affect cell signaling, epigenomic regulation, RNA splicing, protein homeostasis and metabolism (Boveri et al., 2008). Therefore, the search for new active drugs in oncology represents one of the most promising objectives of pharmaceutical industry. Many of these compounds are derived from extraction and purification of toxins and secondary metabolites originating from microorganisms, plants and animals (Adkins et al., 2012).Several compounds from venomous animals, such as snakes, spiders, scorpions, caterpillars, bees, insects and frogs, have largely shown biotechnological or pharmacological applications (Calderon et al.,2014).

Venoms are secretion of venomous animals, synthesized and stored in venom glands. Animals use venoms as a defense mechanism (Gomes et al., 2010). Most of the venoms are complex mixture of biologically active compounds of different chemical nature such as multi-domain proteins, peptides, enzymes, nucleotides, lipids, biogenic amines and other unknown substances.A large number of toxins have been isolated and characterized from snake venoms containing more than 100 proteinaceous toxins. Venom proteins are of biological interest because of their diverse as well as selective pharmacological and physiological effects through their interaction with various molecular targets. Several studies demonstrated the significance of snake and scorpion venom proteins as valuable tools for basic research, disease diagnosis and drug development (Kang et al., 2011).

The present study aimed to evaluate the antiviral as well as the antitumor efficacy of some Egyptian snake and scorpion venoms and related cell death mechanism as there were controversies among authors in regard to the mechanism of action of snake and scorpion venoms on cancer cell lines in vitro.

Materials and Methods:-

Materials:-

Vero cells (African green monkey kidney cells – ATCC No. CCl-181), MRC-5 cells(Normal human lung fibroblast - ATCC No. CCl-171),CACO-2 cells (Human colorectal carcinoma - ATCC No. HTB-37) and MCF-7 cells (Human breast carcinoma - ATTC No. CRL - 2351) were kindly supplied from cell culture department, VACSERA.
VENOMS were kindly supplied from VACSERA animal house, while LAO was purchased from Sigma - Aldrich - USA

Methods:-

Cytotoxicity:-

Evaluation of the cytotoxic effect of test venoms and L-Amino acid oxidase (LAO) was carried out using MTT assay. IC_{50} and percentage viability were calculated using the following equations: (Alley et al., 1988; Ho-Joon et al., 2000)

Number of residual living cells = (OD of treated cells/ OD of untreated cells) X Number of negative control cells (3×10^3 cells/0.1ml).

Percentage viability = (Number of residual living cells / Number of negative control cells) X 100.

In-Vitro anti-cancer activity of test venoms:-

The effect of test venoms on apoptotic genes was performed by extracting RNA from 24 hr venom treated and untreated control cells according to manufacturer's protocol using SV Total RNA Isolation System (Promega-USA). Equal concentrations of the extracted RNA were reversetranscripted to cDNA using Revert Aid first strand cDNA synthesis Kit (Fermantas-Lithuania). The expression of pro-apoptotic genes P53(F: 5'-TCA GAT CCT AGC GTC GAG CCC-3' & R: 5'-GGG TGT GGA ATC AAC CCA CAG-3') &Bax (F: 5'-ATG GAC GGG TCC GGG GAG CA-3' & R: 5'-CCC AGT TGA AGT TGC CGT CA-3') as well as anti-apoptotic gene Bcl-2 (F: 5'-GTG AAC TGG GGG AGG ATT GT-3' & R: 5'-GGA GAA ATC AAA CAG AGG CC-3') was carried out using the newly synthesized cDNA as template for PCR. Semi-quantitative RT-PCR was carried out in triplicates followed by densitometric analysis of band intensities compared to housekeeping gene GAPDH (F: 5'-CAA GGT CAT CCA TGA CAA CTT TG-3' & R: 5'-GTC CAC CAC CCT GTT GCT GTA G-3') using gel documentation system(Marone, 2001).

In- Vivo evaluation of anti-cancer potential of test venoms:-

Mice Inoculation:-

Adult male Swiss albino mice (18-20 g) were injected with 0.1 ml of Ehrlich ascites carcinoma (EAC) cells ($1x10^6$ cells / mice) via S/C and I/P route (5 groups / 10 each) and treated with one type of test venoms (N.haje, N.nigricollis, C.cerastes, Leiurusquinquestriatus scorpion and LAO) by dose resembling half of the LD_{50} of the test venoms (2, 4, 6, 3 and 50 µg/mouse respectively). Two groups were left venom untreated as positive control for S/C route and the other for I/P route. Negative control group was included as well.

Mean Survival Time (MST) and percentage increase in life Span (%ILS):-

The remaining animals were kept for evaluation of survival. Direct injection of test venom combinations was also carried out (Cerastes cerastes, Leiurus scorpion & LAO enzyme) using the safe concentration of each. Antitumor effect of venom was assessed by observing any changes with respect to body weight and ascetic fluid volume.
EAC cell count was determined using trypan blue exclusion technique Boyse et al., (1964). MST of each group containing five mice was monitored by recording the daily mortality for 2 months and ILS (%) was calculated using the following equation (Abu-Sinna et al., 2003).
Mean survival time (MST) = (Day of first death+ day of last death)/2
ILS (%) = (Mean survival time of treated group/ Mean survival time of control group) X100

Viricidal activity:-
Viricidal activity of test venoms was performed according to Aoki and Messiha, (1999), where test viruses were mixed with equal volume of non toxic concentrations as well as 10 fold serial dilutions of test venoms. Negative control viruses were mixed with venom free medium and all sets were incubated at 37°C for 5 min, 2, 6 and 24 hours. Venom treated and untreated viruses were inoculated on 96-well Vero pre-cultured plates and the mean virus titers were determined according to Reed and Muench, (1938).Statistical differences between the mean viral titers were calculated using one way ANOVA. Differences at $p < 0.05$ were considered significant.

Antiviral Activity:-
Pre-cultured 96 well Vero cell plates were treated with the non-toxic concentration of test venoms for 24 hours at 37°C. Test venoms were discarded, plates were inoculated with ten fold serially diluted test viruses. The differences between the mean virus titer in venom treated and untreated cells corresponds to the antiviral activity (Aoki and Messiha, 1999).

Statistical analysis:-
For all experiments, results were represented as the mean ± standard deviation of three independent experiments. Statistical significance was determined using one way ANOVA. Differences at P values less than 0.05 were considered significant.

Results:-

Cytotoxicity:-
Data recorded revealed that Cerastes cerastes venom was the most cytotoxic venom to normal human fibroblast (MRC-5) cells as well as to cancer cell lines (CACO-2 & MCF-7) whereas Leiurus scorpion venom showed the least cytotoxicity to different cell lines as it showed no cytotoxicity to normal human fibroblast (MRC-5) up to 500 µg/ml followed by Naja nigricollis venom, Naja haje venom and LAO enzyme [Table 1]. The cytotoxic effect of test venoms to normal cells (MRC-5) was much lower than its cytotoxicity to cancer cell lines

Table 1: Evaluation of cytotoxicity of test venoms to different cell lines using MTT assay depending on the amount of lactate dehydrogenase releasedfrom residualviable cells 24 hrs post treatment.

Venom	Non toxic concentration (µg/ml ±SD)			
	Vero	**MRC-5**	**CACO-2**	**MCF-7**
Naja haje (NH)	**8** ±0.6	**8** ±0.8	**0.5** ±0.07	**0.200** ±0.005
Naja nigricollis (NN)	**16** ±1.1	**16** ±1.3	**2** ±0.1	**1.00** ±0.1
Cerastes cerastes (CC)	**0.25** ± 0.02	**0.25** ±0.02	**0.05** ±0.003	**0.03** ±0.004
Crude Leiurus (L.crude)	**500** ± 3.1	**500** ±4.2	**8** ±0.6	**8.00** ±0.400
Pure Leiurus (L.pure)	**500** ± 2.8	**500** ±3.1	**8** ±0.5	**8.00** ±0.3
L-Amino acid oxidase (LAO)	**4** ± 0.3	**4** ±0.2	**0.2** ±0.04	**0.050** ±0.003

Evaluation of anticancer activity:-
Recorded data demonstrated that there is a marked up regulation of pro-apoptotic genes (P53 & BAX) post C.cerastes, N. haje, Leiurus venom and LAO enzyme treatment as well as down regulation of anti-apoptotic gene (Bcl-2) 24 hrs post treatment. The densitometric analysis of band intensities revealed that venom treated cells showed a statistically significant increase in p53 as well as Bax/Bcl-2 mRNA expression levels ($P < 0.05$) thus triggering cells towards apoptosis through regulating the expression of these genes [Fig.1&2]

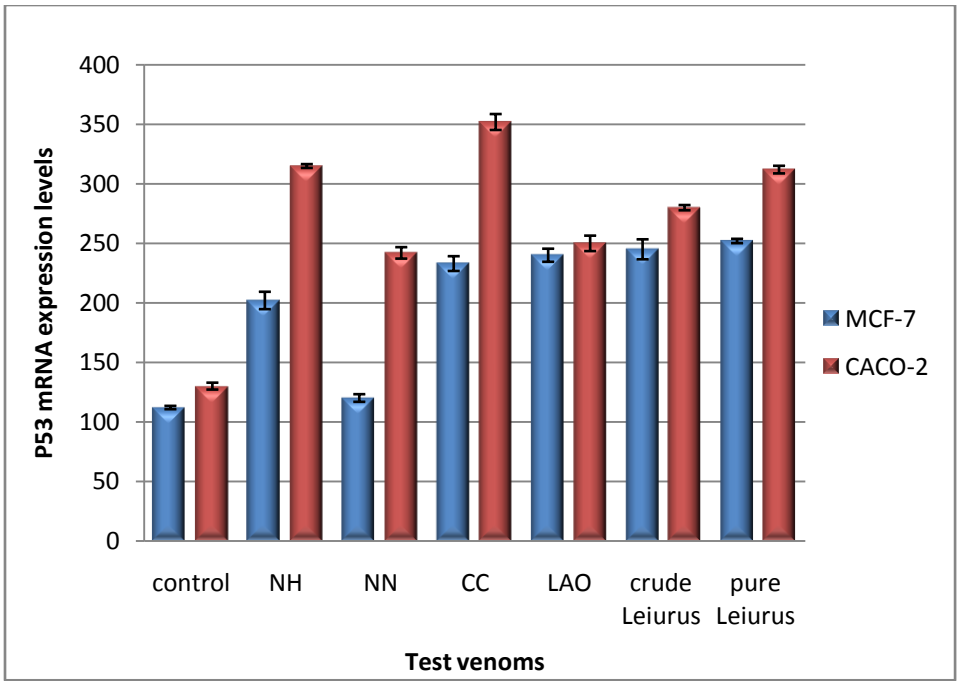


Fig.1: Denstiometric evaluation of p53 mRNA expression levels 24 hrspost treatment with double the non toxic concentration of each venom indicating test venoms induced up-regulation of p53 gene.Recorded values were the mean of three independent tests ± SD

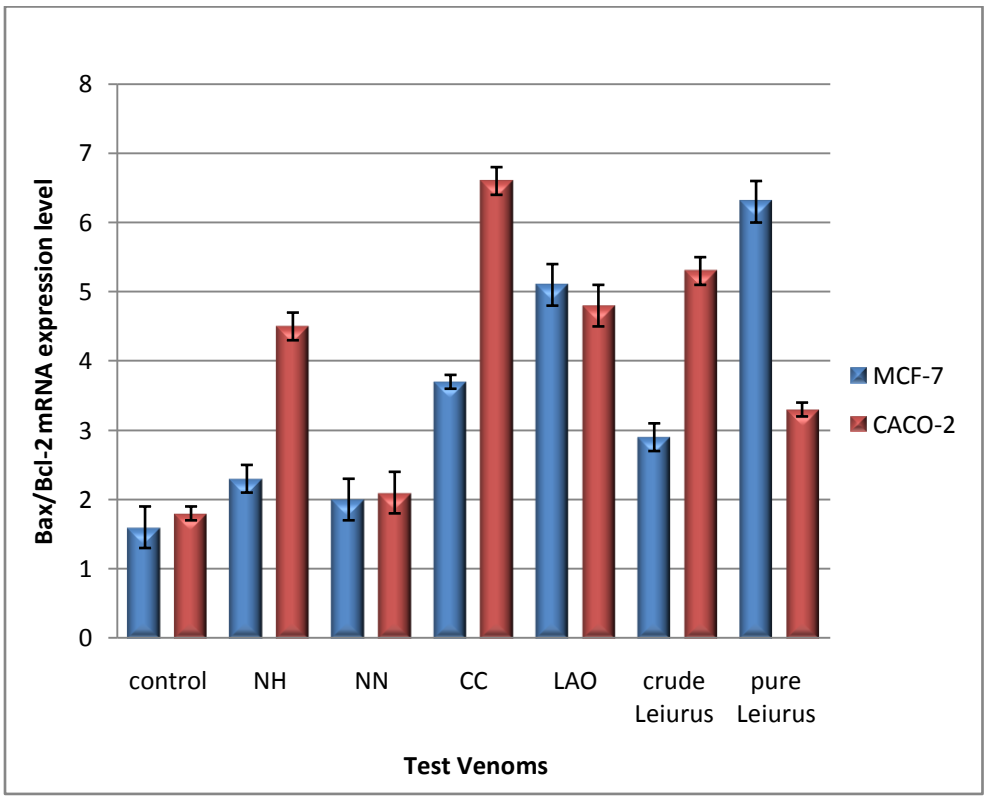


Fig. 2: Denstiometric analysis revealed increased mRNA expression levels of pro-apoptotic gene (Bax) while down regulation of anti-apoptotic gene (Bcl-2). The increased Bax/Bcl-2 mRNA expression ratio demonstrated that test venoms treated cancer cells are directed towards apoptosis

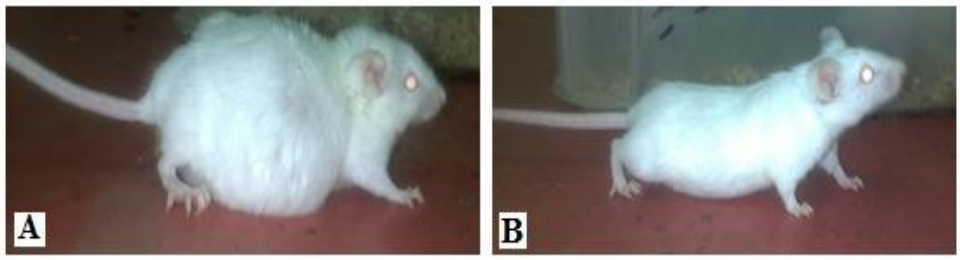
Effect on mice body weight, survival time and ascites volume:-

Recorded data showed that the there was a decreased ascitesin venom treated groups relative tonon treated ones (Images: A-B). The aspirated volume of ascetic fluid was lower in all venom treated groups,where theascetic fluid volumes ranged between 1 to 2.5 ml. Moreover, subcutaneous administration of venoms enhanced the suppression of ascetic fluid development except in case of Naja haje venom treated group which developed a very low volume compared to that of non treated group (6 mL) (Images: C-E). Elevated EAC cell count was predominant in non treated group recording a mean cell count of 154 : 178 $x10^6$ / ml, while a decreased EAC cell count was recorded in all venom treated groups recording 52 : 84 $x10^6$ / ml. It was also obvious that venom treated groups developed a very small mass compared to non treated groups (Images: F-H). In addition, treatment of tumor mass by venom combination (Leiurus scorpion, C.cerastes and LAO enzyme) resulted in very promising results, where the tumor mass markedly decreased post 1st dose of venom combination and seemed to be completely disappeared post the 2nd dose (Images: I-J). Also, there was also a marked increase in the mean survival time of venom treated groups especially those treated with N.nigricollis and C.cerastes venoms compared to that of untreated groups [Table 2].

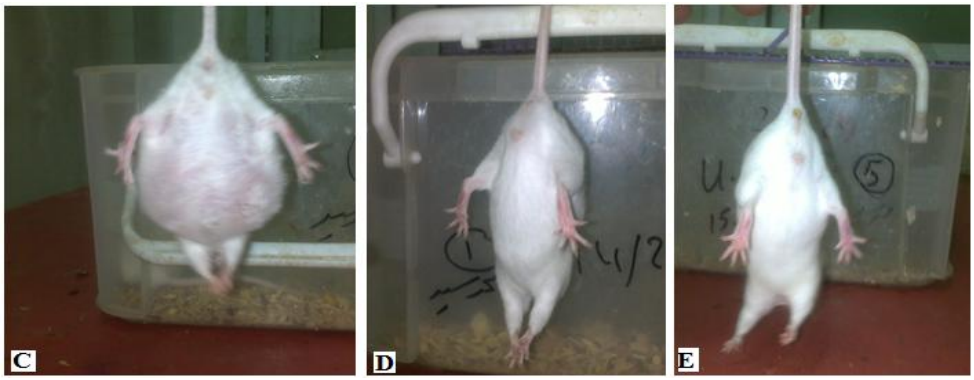
Table 2: Effect of test venoms on mice body weight was evaluated 14 days post EAC cell injectionusing digital balance, peritoneal fluid was aspirated for determining its volume and the total number of EAC cells.

Group	χ BW (g) ±SD	χ AFV (ml)±SD	χ CC(10^6/ml) ±SD	χ ST (%)
EAC (S/C)	**19±1.5**	**6±0.27**	**154±8.6**	100
EAC (S/C) + Naja haje	**22±1.1**	**1±0.13**	**68±4.5**	100
EAC (S/C) + N. nigricollis	**24±1.4**	--	--	350
EAC (S/C) + C.cerastes	**24.5±0.9**	--	--	250
EAC (S/C) + Leiurus	**23±1.8**	--	--	200
EAC (S/C) + LAO	**22.5±1.9**	--	--	160
EAC (I/P)	**16±0.7**	**7±0.43**	**178±11.8**	100
EAC (I/P) + Naja haje	**21±0.9**	**2.5±0.11**	**76±5.8**	100
EAC (I/P) + N. nigricollis	**23.5±0.8**	**1±0.11**	**80±3.6**	200
EAC (I/P) + C.cerastes	**23±1.8**	**1±0.13**	**52±2.2**	200
EAC (I/P) + Leiurus	**21.5±1.1**	**2±0.17**	**84±7.1**	100
EAC (I/P) + LAO	**21±1.2**	**1.5±0.14**	**78±2.9**	190
(-ve control)	25±1.7	----	----	----

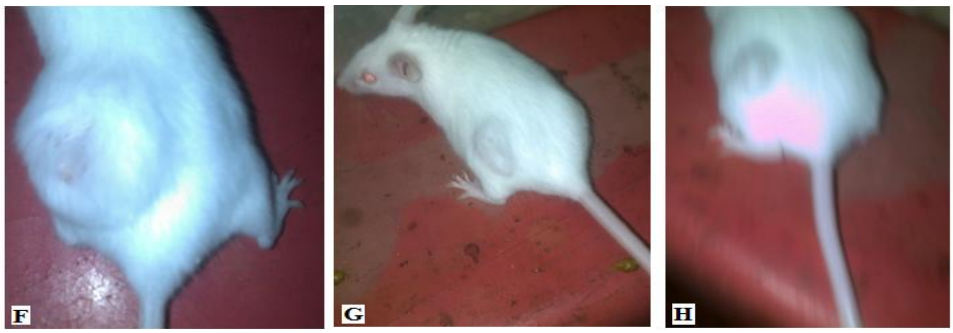
PI: post injection, BW: Body weight (Body weight determined 14 days post injection), ST: Survival time, CC: Cell count, AFV: Ascetic fluid volume



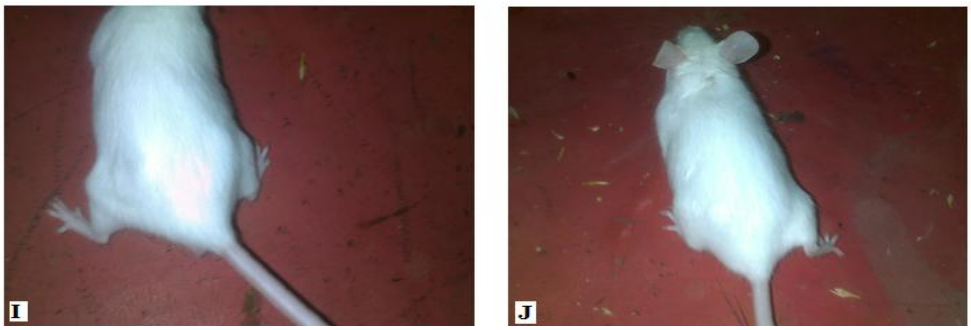
A: Mice with induced ascites using EAC cells (untreated).
B: Mice of venom treated group.



C: Ascites in non treated mice; **D:** Ascites inNaja nigricollis venom treated mice;**E:**Ascites in C.cerastes venom treated mice (14 days post treatment)



F: Tumor mass developed in non treated mice; **G:** Tumor mass in mice treated with Naja nigricollis venom; H:Tumor mass in mice treated with C.cerastes venom (14 days post treatment)



I:Tumor mass after local treatment with venom combination (post 1st dose);
J: Tumor mass after local treatment with venom combination (post 2nd dose)

Viricidal activity:-

Data recorded revealed that test venoms exhibited potential viricidal activity against viral models at different time intervals post treatment, where VSV titer was decreased by 0.3- 0.9 $\log_{10}$, 5 minutes post treatment representing 5 - 15% of the initial titer. Virus titer was also reduced relatively to time till complete reduction of virus titer (6 hrs post treatment) in all test venoms except in case of Cerastes cerastes venom, where the reduction rate of VSV were 75% & 100%, post treatment by 6 & 24 hrs respectively [Fig3].

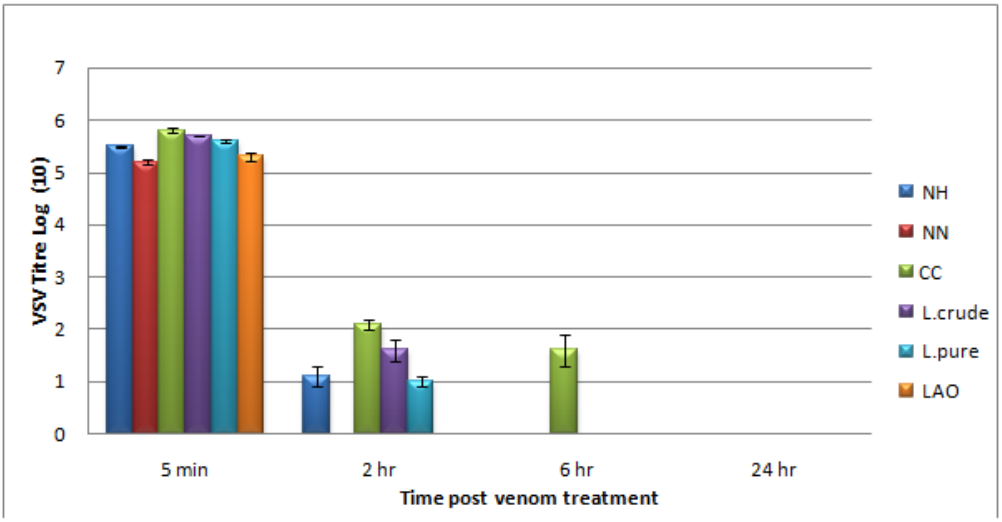


Fig 3: Viricidal activity of test venoms against VSV, where the virus was incubated with different test venoms and the virus titre was evaluated at different time intervals. A marked time dependent viral titre reduction was observed till complete depletion in the virus titre 24 hrs post treatment

Regarding viricidal activity against HSV-II, it was found that all venoms reduced the virus titer post virus treatment at different durations, where the virus titer was reduced by 0.3 - 0.5 $\log_{10}$ / ml (5 - 8% of the initial titer); the reduction was time related, where the depletion rate was in the order of 2.5 - 3.5 $\log_{10}$ / ml and 3-4 $\log_{10}$ / ml(40 - 55% and 50-56 %) 2 and 6 hrspost treatment. The viricidal activity of different venoms 24 hours post treatment reaches 3.5 - 5 $\log_{10}$ / ml; 55 - 80% of the initial titer [Fig4]. Also, upon determining the effect of sub-non toxic concentrations of test venoms on both viruses, it was demonstrated that the exhibited viricidal activity was concentration dependent [Table 3].

Venom	Venom conc.	VSVχVTR $\log_{(10)}$ 6hr PT	VSV χ VTR % 6hr PT	HSV χ VTR $\log_{(10)}$ 6hr PT	HSV χ VTR % 6hr PT
Naja haje	8 µg/ml	**6.1** ±0.2	**100**	**4** ±0.2	**65**
	4 µg/ml	4.9 ±0.3	80	3.3 ±0.2	53
	2 µg/ml	2.1±0.1	34	1.4±0.1	23
Naja nigricollis	16 µg/ml	**6.1** ±0.1	**100**	**3** ±0.2	**50**
	8 µg/ml	5±0.5	82	2.5±0.1	40
	4 µg/ml	2.2±0.2	36	1.3±0.1	21
Cerastes cerastes	0.25µg/ml	**4.5** ±0.4	**75**	**3** ±0.2	**50**
	0.1 µg/ml	4±0.3	65	2.4±0.2	38
	0.05µg/ml	1.7±0.1	28	1±0.1	16
Crude Leiurus	500µg/ml	**6.1** ±0.2	**100**	**4** ±0.4	**65**
	250µg/ml	4.8 ±0.1	79	3.4±0.3	55
	125µg/ml	2.3±0.1	38	1.3±0.1	21
Pure Leiurus	500µg/ml	**6.1** ±0.3	**100**	**4** ±0.2	**65**
	250µg/ml	4.9 ±0.2	80	3.2±0.1	51
	125µg/ml	2.6 ±0.2	43	1.3±0.1	21
L. amino oxidase	4 µg/ml	**6.1** ±0.4	**100**	**4** ±0.1	**65**
	2 µg/ml	5.1 ±0.4	84	3.3±0.2	53
	1 µg/ml	2.5±0.2	41	1.5±0.2	24

χ VTR: mean virus titer reduction
PT: Post treatment

Table 3: Viricidal activity of sub-non toxic concentrations of test venoms depending on determining the mean virus titer reductionas well as the percentage reductioncompared to negative control. Data revealed a concentration dependent decrease in the virus titre6 hrs post treatment

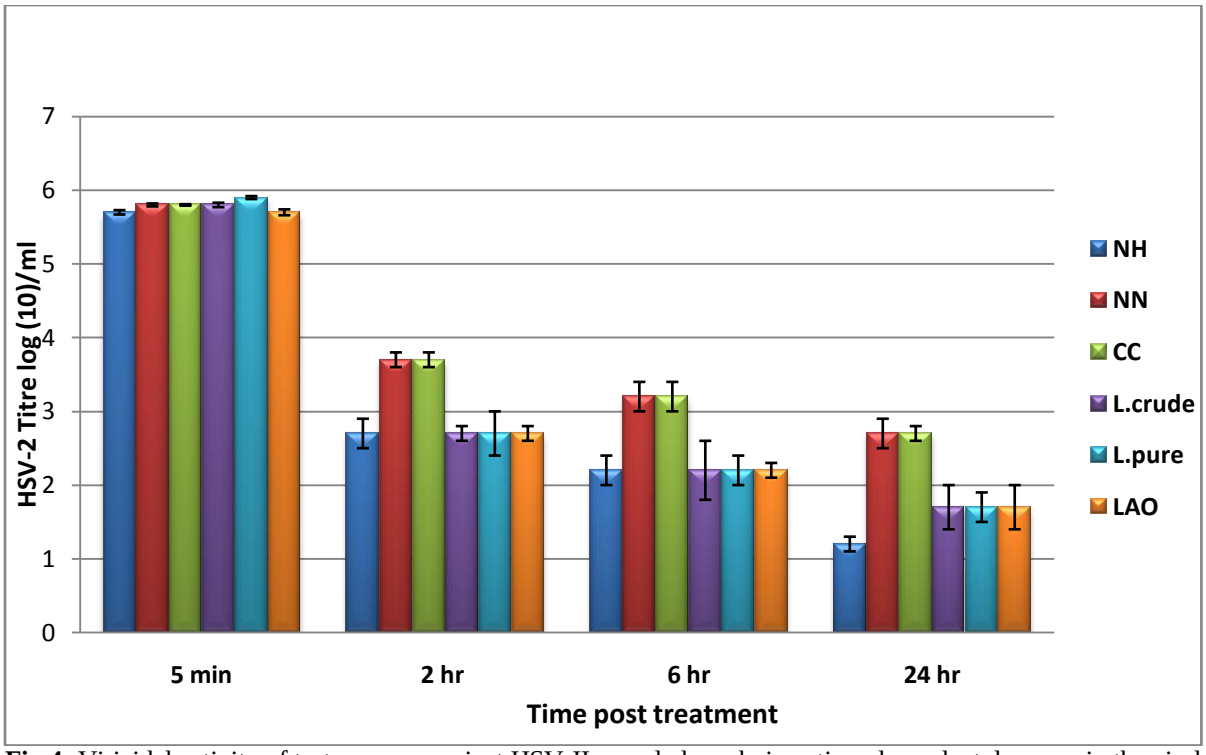


Fig 4: Viricidal activity of test venoms against HSV-II revealed an obvious time dependent decrease in the viral titre post venom treatment.

Antiviral activity:-

Data recorded revealed that test venoms reduced the titre of both viruses in 24 hrs venom pretreated cells recording 0.6 - 2 $\log_{10}$and 1 - 2.5 $\log_{10}$ reduction in case of VSV&HSV-II respectively. Naja nigricollis was the most effective venom against VSV where it decreased the virus titer by 30%. On the other hand, Leiurus scorpion venom was the most effective venom against HSV- II either crude or pure form; as it reduced HSV- II titer by 40%. [Fig5]

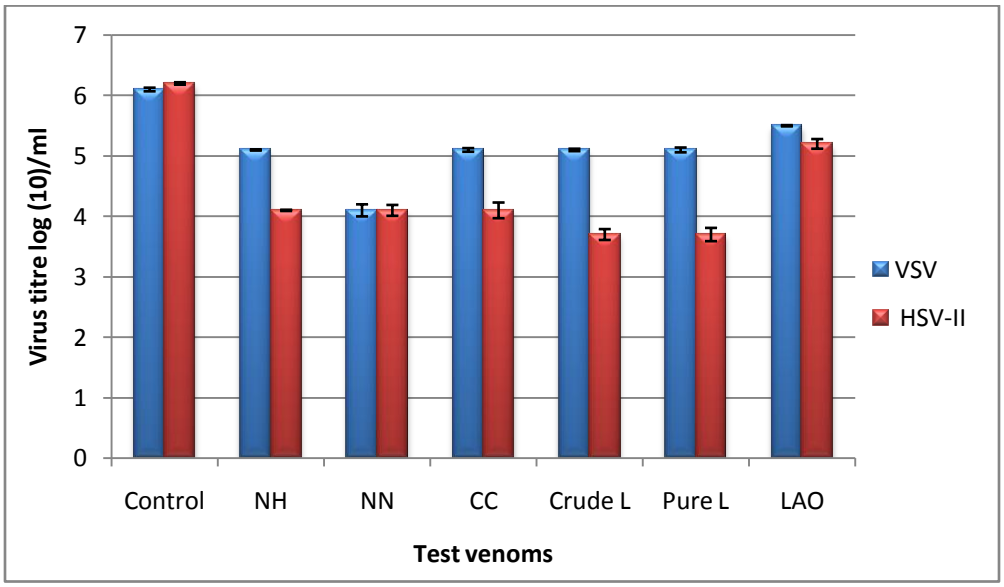


Fig 5: Antiviral activity of test venoms against VSV and HSV-II, where the virus titre was determined in 24 hr venom pretreated Vero cells compared with its tire in untreated cells. The recorded difference in viral titre indicates that test venoms showed a statistically significant antiviral activity.

Discussion:-

Snake venoms proteins bioactivity supported the discovery and development of new therapeutic agents. Also, they were used in the diagnosis of several diseases as well as they were considered as a valuable tool in basic research (Nair et al., 2007).The first successful example of developing a drug from a snake venom toxin was the anti-hypertensive drug (captopril), an angiotensin-converting enzyme inhibitor from Bothropsjararaca venom (Koh and Kini, 2012). It was also reported that anticancer therapy is an important area for the application of proteins and peptides derived from venomous animals where it plays multiple important roles in cancer pathology including tumor cell proliferation, angiogenesis, invasion and metastasis (Goodman and Picard, 2012).

Therefore, test venoms were investigated for their cytotoxicity to human colorectal and breast cancer cell lines (CACO-2 and MCF-7) using MTT assay and it was recorded that all test venoms showed an obvious cytotoxic effect to cancer cell lines which was extremely exceeding that to normal cell line (MRC-5). Data recorded was in alignment with that ofCarvalho et al., (2001)who reported that B. jararacussu venom was a potential inhibitor to the proliferation of some tumor and endothelial cell lines. In another study, it was demonstrated that cytotoxin isolated from Naja naja venom was cytotoxic to Yoshida sarcoma cells implanted in mice, but was non toxic to the experimental animals(Braganca et al., 1967). It was also reported that atroporin and kaotree derived from Crotalusatrox and Naja n. kaouthia venoms had anti-proliferative effect to some human breast, colon, liver, and ovary as well as animal cancer cell lines at concentrations as low as 0.5 µg/ml, in addition to their synergetic activity as anti-proliferating to cancer cells but not to normal cells (Lipps, 1999). Recently, PLA_2 derived from Bothropspauloensis venom was demonstrated to induce cell death in Human breast cancer cells(Azevedo et al., 2016). In addition, anticancer activity of scorpion venom against colorectal and breast cancer cell lines was also reported (Al-Asmari et al.,2016).

Several authors have reported the anti-cancer potential of snake venom while exact mechanism of venom is not fully understood so a continuous research is urgently required (Abu-Sinna et al., 2003). One of the major defects in cancer was the resistance of cancer cells to undergo apoptosis due to malfunction of molecules like Bcl-2 (Haldar et al., 1995), Ras(White et al., 1995), telomerase (Shay and Bacchetti, 1997), p53(Soengas et al., 1999) and Caspases(Schotte et al., 2001). As a consequence apoptosis regulation in both normal and malignant cells has become an area of extensive study in cancer research (Johnstone et al., 2002).

In agreement with results obtained in this study it was found that venom inducedcytotoxic effect may be mediated by alterations in apoptotic gene regulation(Torii et al., 1997; Abe et al., 1998; Reinaldo et al., 2002;Correa et al., 2002). Moreover, L-amino acid oxidase enzyme associated apoptotic processes may be involved in the growth inhibition of the tumor cells(Raibekas and Massey, 1998).Another study demonstrated thatLeiurusquinquestriatusscorpion venom exhibited apoptotic effect on 293T and C2C12 cell lines in different concentrations(Omran, 2003).Snake venom may also inhibit cancer progression in laboratory animals(Zhou et al., 2000; Hantgan et al., 2005).Cytotoxicity records were also supported on small cell lung cancer cells (SCLC)(Tang et al., 2003;Poulsen et al., 2005).

The ratio of Bax/Bcl-2 might be a critical factor of a cell's threshold for undergoing apoptosis wherehigh Bax/Bcl-2 ratio was an indicator of greater apoptotic activity. As a result this ratio could aid in evaluating the sensitivity of a cancer cell to treatment(Chipuk and Green, 2008; Zaldivar et al., 2009; Lee et al., 2012). LAO was also reported to exhibit cytotoxicity against human tumorigenic cells through an increase in caspase-8 and -9 activities (Fung et al.,2015). In another study, it was reported that LAO induce apoptosis in human breast cancer (MCF-7) cells by caspase-7 activation and reactive oxygen species production through the generation of high amounts of H_2O_2(Mukherjee et al.,2015).

Antitumor efficacy of test venoms was evaluated In-Vivo,mice body weight and mean survival time were determined post treatment revealing obvious anticancer potentials for test venoms, where a marked decrease in the size of tumor mass was recorded post systemic treatment while tumor masses tend to completely disappeared post treatment with venom combinations. These findings were also accompanied with decreased ascetic fluid volume, EAC count and body weight as well as survival time prolongation in venom treated mice.

It was reported in agreement with previous results that cobra venom could be used as a source of useful biological active compounds (Mohamed and Omran, 2003)that it might present a clue to treat various tumors either used alone or in combination with drugs(Hantgan et al., 2005). In addition, the prolongation of life span of treated animals, decrease in tumor volume as well as decrease in EAC cell count could be considered as reliable criteria for assessing

the potential of anticancer agents, where an obvious decrease in EAC cell count and prolongation in the life span by 52.3% was recorded post treatment with Echis coloratus crude venom(Mady,2002).Another study also demonstrated the anti-tumor efficacy of crude Macrovipera lebetina venom in S-180 sarcoma bearing mice (Ghazaryan et al.,2015).

Concerning the antiviral activity, test venoms were examined for its antiviral and viricidal activity against a DNA and RNA virus models (HSV-II and VSV). Results revealed very promising records for all test venoms and recorded variable inactivation potential for different viruses with more viricidal potentials compared to antiviral ones. In agreement with our study, it was reported that snake venoms biomolecules have great antiviral potentials (Mion et al., 2002; Ammendolia et al., 2007).Non-cytotoxic venom fractions from Crotalusdurissusterrificus (Cdt) inhibited Measles virus replication in Vero cells either prior to or during viral infection(Petricevich and Mendoça, 2003). Phospholipase A_2 (PLA_2) venom derivative was found to exhibit antiviral activity against HIV(Villarrubia et al., 2004)suggesting that it may block the viral entry into target cell (Singla and Garg, 2013).PLA_2 was also reported to exhibit antiviral activity against Dengue virus (Cecilio et al.,2013) by disturbing its viral envelope(Muller et al., 2014). Another study demonstrated that Naja siamensis snake venom derivative inhibited the infection of lymphocytes by HIV (Shivaji, 2007).Moreover,BothropsjararacavenomderivedLAO showed antiviral activity against Dengue virus with 83 fold reduction in viral titre compared to untreated cells(Sant'Ana et al., 2008).

LAO derived fromTrimeresurusstejnegeri, Crotalusatrox and Pseudechis australis snake venoms could inhibit infection and replication ofHIV (Du &Clemetson, 2002), where this activity might be due to production of reactive oxygen species (Zhang et al., 2003).Other studies conducted using different snake venoms such as Crotalusadamanteus, Oxyuranusmicrolepidotus, Bungaruscandidus, Hydrophiscyanocinctus, Naja naja, Notechisaterater, Naja sumatrana and Naja kaouthia have demonstrated anti-HIV activities for these venoms (Nair et al., 2007).Mucroporin, scorpion venom derivative, was reported to exhibit antiviral activity against measles and influenza virus (H5N1) due to interaction with the viral envelope (Li et al., 2011) as well as the viricidal activity of Egyptian scorpion venoms against hepatitis C virus(El-Bitar et al., 2015).

The present study highlights the role of test venoms in triggering cancer cells towards apoptosis through up-regulation of pro-apoptotic genes as well as down regulation of anti-apoptotic genes with minimal effect on normal cells. Potential time dependent viricidal activity was obviousagainst VSV and HSV-II. In addition, antiviral activity against test viruses in venom pretreated cells suggested that test venoms may also interfere with the initial stages of virus replication in the host cells.

Accordingly, the promising anticancer potentials of snake and scorpion venoms as well as their derivatives encouraged us to intensify our studies to use specified purified venom derivatives as a new approach toenhance their potentials using different formulations either via nanocapsulationwith biodegradable molecules with respect to evaluation of the long lasting effect and related therapeutic and physiological drawbacks.Also their marked viricidal potentials may be examinedas viral inactivant in vaccine production.

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