RESULTS

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This study included 45 patients, all of them were infants and children < 5 years old suffering from symptoms and signs of acute respiratory tract infections such as pneumonia, bronchiolitis & bronchopneumonia and 10 children were free from symptoms and signs of chest infection at the same age group were served as control group.

I- Results of history and clinical data :

Table (1): Comparison between cases and controls as regards age.

Studied group / Age (years)	Cases (n = 45)	Controls (n = 10)	Total (n = 55)
Range	0.17 – 4.0	0.083 – 1.5	0.083 – 4.0
X	0.81667	0.4833	0.75606
± SD	± 0.69998	± 0.4936	± 0.6758
t	1.776		
p	> 0.05		

This table shows that there was no significant difference between cases & controls as regards age (P > 0.05).

Table (2): Respiratory syncytial virus infection [as diagnosed by direct immunofluorescence (DIF) from nasopharyngeal aspirate (NPA)] versus age of the patients.

DIF / Age (years)	+ ve (n = 34)	- ve (n = 11)	Total (n = 45)
Range	0.17 – 1.67	0.25 – 4.0	0.17 – 4.0
X (Mean)	0.7403	1.0527	0.81667
± SD	± 0.5398	± 1.0551	± 0.69998
t	0.943		
p	> 0.05		

This table shows no significant relation between RSV infection and the age of patients : 34 (75.56%) out of 45 children were RSV positive by DIF. their age ranged from (0.17 – 1.67) years with mean 0.7403. 11 (24.44%) out of 45 children were RSV negative by DIF. their age ranged from 0.25 – 4.0 years with mean 1.0527.

Table (3): Distribution of RSV infection among children with different age groups.

DIF / Age (in years)	+ ve		- ve		Total	
	No.	%	No.	%	No.	%
< 1	28	80.0	7	20.0	35	100.0
1 – 2	5	62.5	3	37.5	8	100.0
2 +	1	50.0	1	50.0	2	100.0
Total	34	75.56	11	24.44	45	100.0

This table shows that:

- Twenty eight (80%) out of 35 children with age less than 1 years were RSV positive by (DIF) direct immunofluorescence from nasopharyngeal aspirate (NPA).
- Five (62%) out of 8 children with age ranged between 1-2 years were RSV positive by DIF from (NPA).
- One (50%) out of 2 children with age > 2 years was RSV positive by DIF from (NPA).

Table (4): Distribution of cases & controls according to sex.

Studied group / Sex	Cases		Controls		Total	
	No.	%	No.	%	No.	%
Female	25	55.6	5	50.0	30	54.5
Male	20	44.4	5	50.0	25	45.5
Total	45	100.0	10	100.0	55	100.0
Z	0.286		0.136		———	
P	> 0.05		> 0.05		———	

This table shows no significant difference (P > 0.05) between cases and controls as regards their sex.

- Twenty five (55.6%) out of 45 cases were females and 20 (44.4%) were males.
- Five (50%) out of 10 controls were female & 5 (50%) were males.

Table (5): Respiratory syncytial virus infection [as diagnosed by direct immunofluorescence (DIF) from nasopharyngeal aspirate (NPA)] versus sex of the patients.

DIF / Sex	+ve		-ve		Total	
	No.	%	No.	%	No.	%
Female	18	72.0	7	28.0	25	100.0
Male	16	80.0	4	20.0	20	100.0
Total	34	75.56	11	24.44	45	100.0
Z	0.549		0.305		———	
P	> 0.05		> 0.05		———	

This table shows no statistically significant difference (P > 0.05) between males and females as regards RSV infection. 18 (72%) out of 25 female patients were RSV positive by DIF and 16 (80%) out of 20 male patients were RSV positive by DIF.

Table (6): Respiratory syncytial virus RSV infection [as diagnosed by direct immunofluorescence (DIF) from nasopraryngeal aspirate (NPA)] versus different clinical diagnosis of patients.

DIF / Clinical diagnosis	+ ve		- ve		Total	
	No.	%	No.	%	No.	%
Bronchiolitis	26	86.67	4	13.33	30	100.0
Pneumonia	3	75.0	1	25.0	4	100.0
Bronchopneumonia	5	45.45	6	54.55	11	100.0
Total	34	75.56	11	24.44	45	100.0
X =	7.402					
P	< 0.05					

This table shows that:

- RSV was significantly higher in patients with bronchiolitis than in patient with pneumonia or bronchopneumonia ($P < 0.05$).
- Twenty six (86.67%) out of 30 patients with bronchiolitis were RSV positive by DIF .
- Three (75%) out of 4 patients with pneumonia were RSV positive by DIF and 5 (45.45%) out of 11 patients with bronchopneumonia were RSV positive by DIF.

II – Results of Immunofluorescence and cell culture :

Table (7): Direct immunofluorescence (DIF) technique for detection of RSV antigen [in nasopharyngeal aspirate samples (NPA) versus cell culture].

Cases (n =45) / Test.	+ve		-ve		Z*	P
	No.	%	No.	%		
DIF from NPA	34	75.56	11	24.44	0.409	> 0.05
DIF after cell culture	32	71.11	13	28.99		

Z* : Result bet. Positive

This table shows :

- No significant difference between DIF from NPA & DIF after cell culture in detecting RSV antigen (P > 0.05).
- Thirty four (75.56%) out of 45 patients were RSV positive by DIF from NPA & 32 (71.11%) were RSV positive by DIF after cell culture.

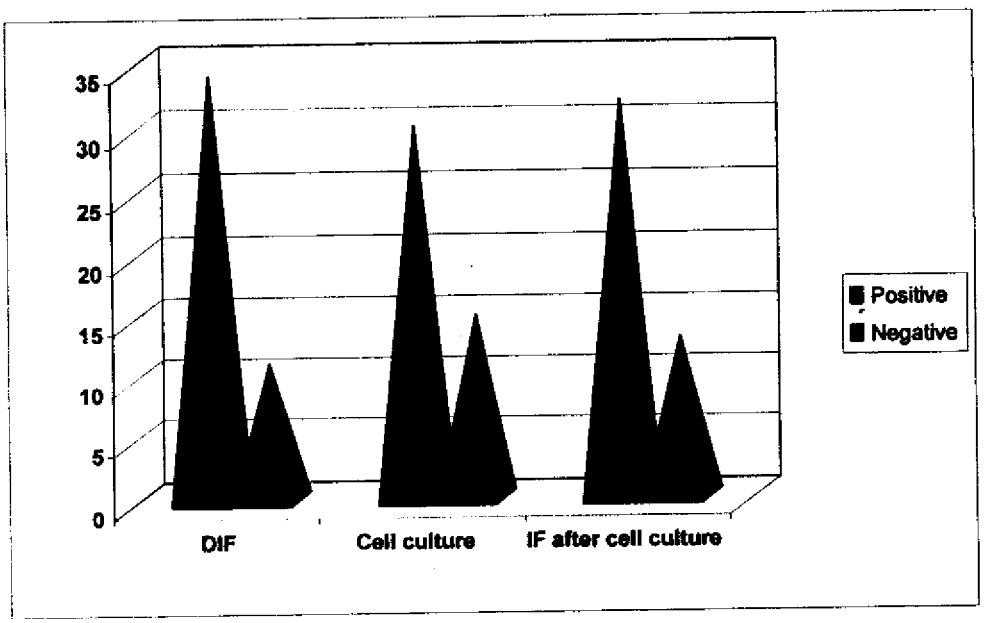
Table (8): Distribution of cases & controls according to direct immunofluorescence (DIF) from nasopharyngeal aspirate (NPA), cell culture result and direct immunofluorescence (DIF) after cell culture.

Studied groups / Test	Cases (n = 45)				Controls (n = 10)			
	+ ve		- ve		+ ve		- ve	
	No.	%	No.	%	No.	%	No.	%
DIF from NPA	34	75.56	11	24.44	0	0.0	10	100.0
Cell culture	30	66.67	15	33.33	0	0.0	10	100.0
DIF after cell culture	32	71.11	13	28.99	0	0.0	10	100.0

This table shows that :

- Thirty four (75.56%) out of 45 cases were RSV positive by DIF from NPA samples, 32 (71.11%) were RSV positive by cell culture & 30 (66.67%) were RSV positive by DIF after cell culture.
- Non of the control group showed positive test for RSV infection by DIF from NPA samples, cell culture or DIF after cell culture.

Fig. (1): Distribution of cases according to direct immunofluorescence (DIF), from NPA samples cell culture result and direct immunofluorescence (DIF) after cell culture.



Table(9): Validity of direct immunofluorescence (DIF) from nasopharyngeal aspirate NPA samples as a screening test assuming that direct immunofluorescence (DIF) after cell culture is the confirmatory test].

Validity of DIF test	Among females	Among Males	Among Total
Sensitivity	100.0%	93.33 %	100.0%
Specificity	100.0%	66.67 %	84.62 %
+ ve predictivity	100.0%	87.5 %	94.12%
- ve predictivity	100.0%	100.0%	100.0%
Accuracy	100.0%	95.0%	95.56 %

This table shows that : The sensitivity of DIF test in detecting RSV antigen is 100.0% and the specificity is 84.62%.

Table (10): Validity of direct immunofluorescence (DIF) from nasopharyngeal aspirate (NPA) samples as a screening test assuming that isolation on cell culture is the confirmatory test

Validity of DIF test	Among females	Among Males	Among Total
Sensitivity	100.0%	100.0 %	100.0 %
Specificity	100.0%	66.67 %	68.75 %
+ ve predictivity	83.33 %	87.5 %	85.92 %
- ve predictivity	100.0%	100.0%	100.0 %
Accuracy	88.0 %	90.0%	88.89 %

This table shows that: The sensitivity of DIF test in detecting RSV antigen is 100.0% and the specificity is 68.75%.

Table (11): Validity of cell culture as a screening test assuming that direct immunofluorescence (DIF) after culture is the confirmatory test.

Validity of cell culture	Among females	Among Males	Among Total
Sensitivity	83.33%	100.0 %	90.63%
Specificity	100.0%	100.0 %	100.0 %
+ ve predictivity	100.0%	100.0 %	100.0%
- ve predictivity	70.0%	100.0%	81.25%
Accuracy	88.0%	100.0%	93.33 %

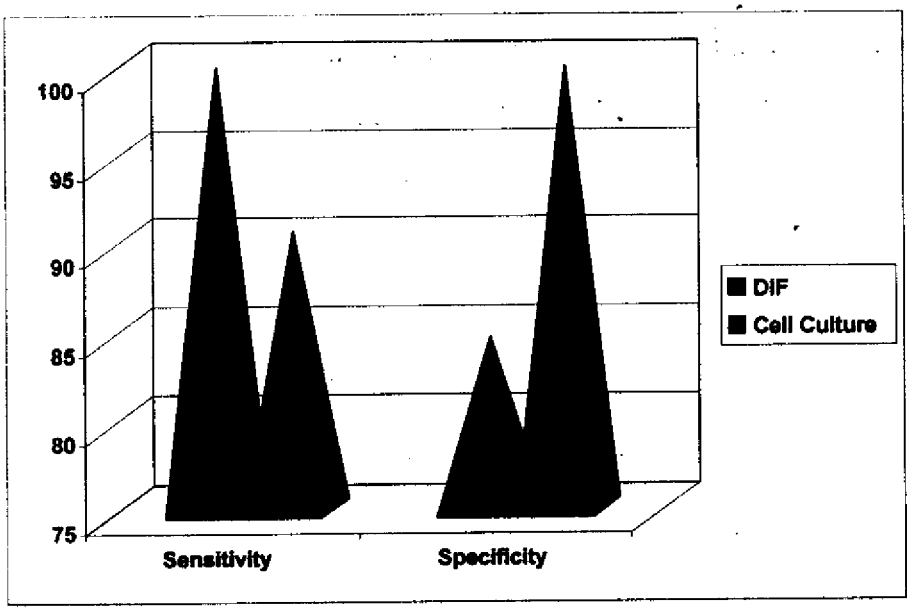
This table shows that : The sensitivity of cell culture technique in detecting RSV infection is 90.63% and the specificity is 100.0%.

Table (12): Sensitivity of cell culture versus direct immunofluorescence (DIF) from nasopharyngeal aspirate (NPA) samples.

Test	Sensitivity	Specificity
DIF from NPA	100.0 %	84.62 %
Cell culture	90.63 %	100.0%

This table shows that : DIF is the most sensitive test and cell culture is the most specific test for detection of RSV infection.

Fig. (2): Sensitivity of cell culture versus direct immunofluorescence (DIF) from NPA samples.



III – Results of Apoptosis :

Table (13): Assessment of apoptosis in RSV infected HEP-2 cells (after 48 hrs).

Cell culture / Apoptosis	+ve (n = 30)		-ve (n = 15)		P
	No.	%	No.	%	
+ ve	0	0.0	0	0.0	> 0.05
- ve	30	100.0	15	100.0	

This table shows that:

- The occurrence of apoptosis in RSV infected HEP-2 cells was statistically non significant (P > 0.05).

- Non of 30 RSV infected cells showed apoptotic changes either by Giemsa staining or by DNA electrophoresis after 48 hrs post RSV inoculation.

Table (14): Assessment of apoptosis in RSV infected HEP-2 cells (after . 72 hrs).

Cell culture / Apoptosis	+ve (n = 30)		-ve (n = 15)		P
	No.	%	No.	%	
+ ve	6	20.0	0	0.0	> 0.05
- ve	24	80.0	15	100.0	
Total	30	100.0	15	100.0	

This table shows that:

- The occurrence of apoptosis in RSV infected HEP-2 cells was statistically non significant (P > 0.05).

- Six (20%) out of 30 RSV infected cells showed apoptotic changes both by Giemsa staining & by DNA electrophoresis.

- Non infected cells showed No apoptotic changes.

Table (15): Apoptosis versus diseases caused by RSV infection.

Apoptosis / Diseases	+ve		- ve		Total	
	No.	%	No.	%	No.	%
Bronchiolitis	5	22.72	17	77.28	22	100.0
Pneumonia	1	33.33	2	66.67	3	100.0
Bronchopneumonia	0	0.0	5	100.0	5	100.0
Total	6	20.0	24	80.0	30	100.0

This table shows that :

- Five (22.72%) out of 22 patients with RSV bronchiolitis showed apoptosis of the infected cells.

- One (33.33%) out of 3 patients with RSV pneumonia showed apoptosis in infected cells.

Fig. (3): Apoptosis versus diseases caused by RSV infection..

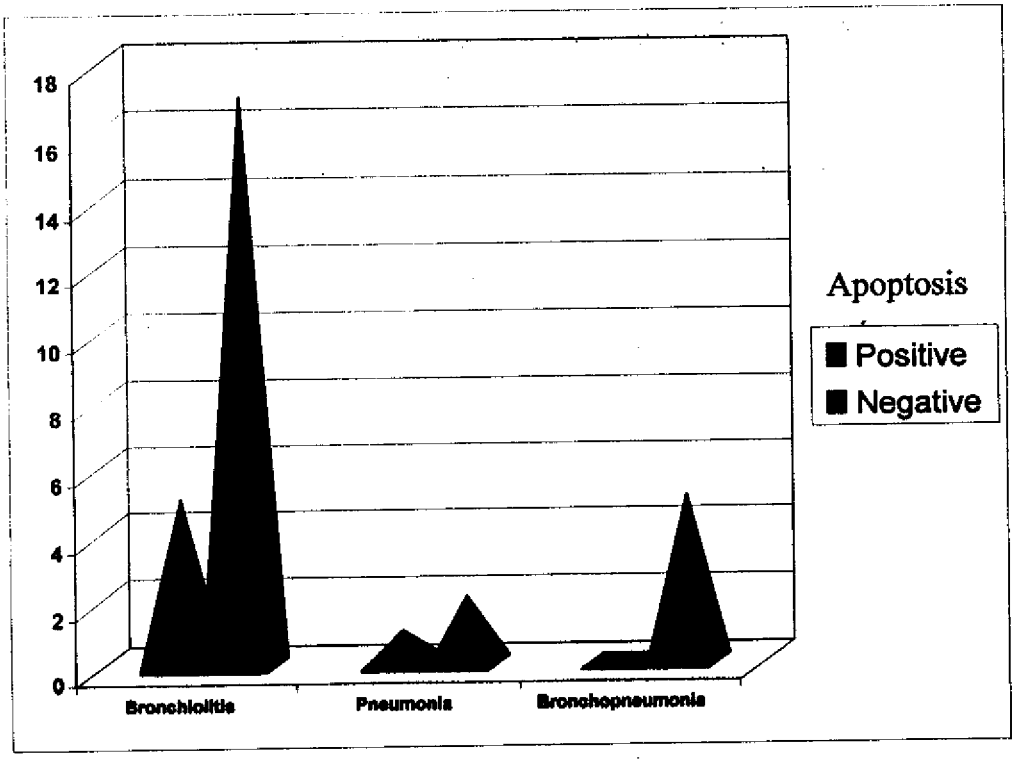


Table (16): Apoptosis versus severity of clinical picture (respiratory distress).

Apoptosis / Resp. distress	+ve		- ve		Total	
	No.	%	No.	%	No.	%
+ ve	0	0.0	22	100.0	22	100.0
- ve	**6**	**50.0**	**6**	**50.0**	**12**	100.0
Total	6	17.65	28	82.35	34	100.0
Z	2.449		2.449		———	
P	< 0.05		< 0.05		———	

This table shows that :

- Apoptosis was significantly high in patients with mild clinical picture (No respiratory distress).

- Non of RSV isolated from patients with respiratory distress were able to induce apoptosis in HEP-2 cells.

Table (17): Giemsa stain versus DNA electrophoresis as two methods for assessment of apoptosis.

Test / Apoptosis	Geimsa stain		DNA electrophoresis	
	No.	%	No.	%
+ ve	6	13.33	6	13.33
- ve	39	86.77	39	86.77
Total	45	100.0	45	100.0
P	> 0.05			

This table shows that : There was no significant difference between Giemsa stain & DNA electrophoresis in assessment of apoptosis ($P > 0.05$).

Fig. (5): Normal HEP-2 cells stained by Giemsa stain.

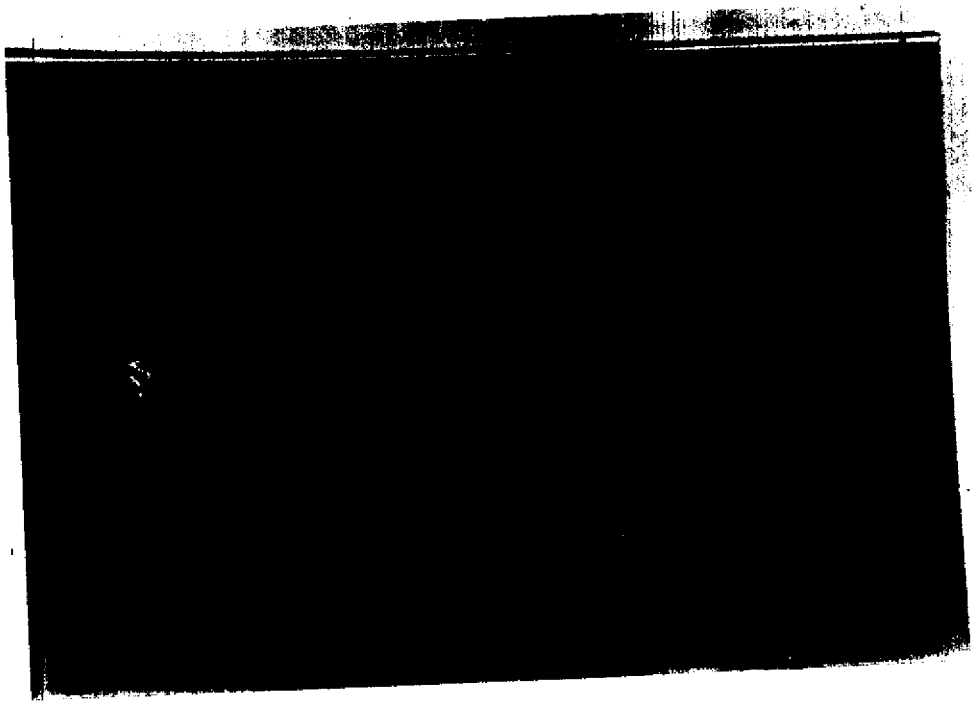


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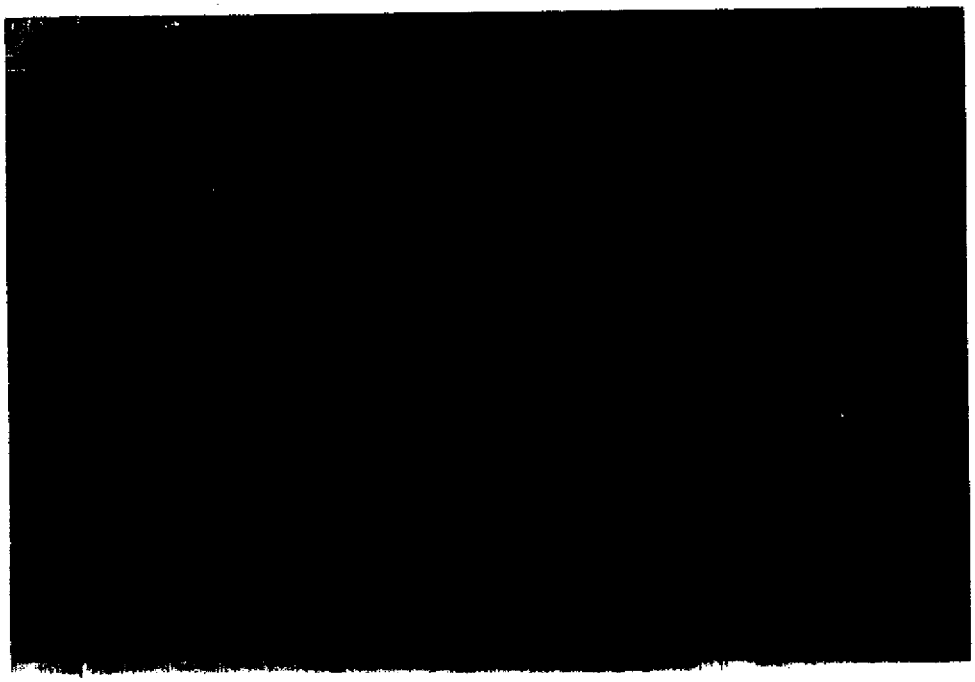
Fig. (6): HEP-2 cells infected with RSV and stained by Giemsa stain showing multinucleated cells (syncytium).



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:. (7): Morphological changes of apoptosis in RSV infected HEP –2 cells.

gnification power 1000 x

ccentric nucleus

)ense chromatin

.poptotic bodies

Fig. (8): RSV infected cells (Positive DIF test).

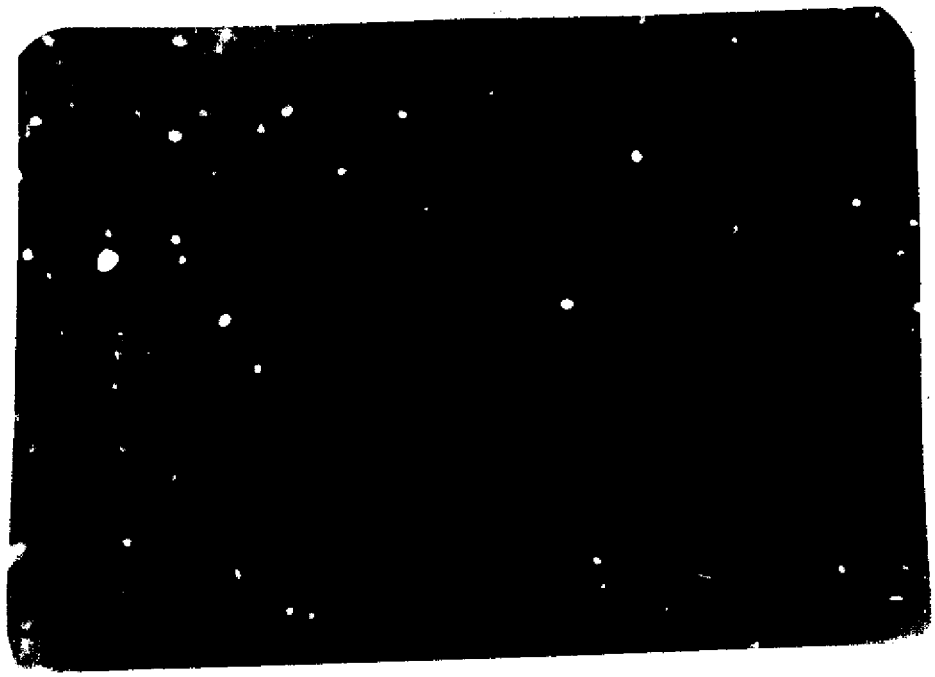

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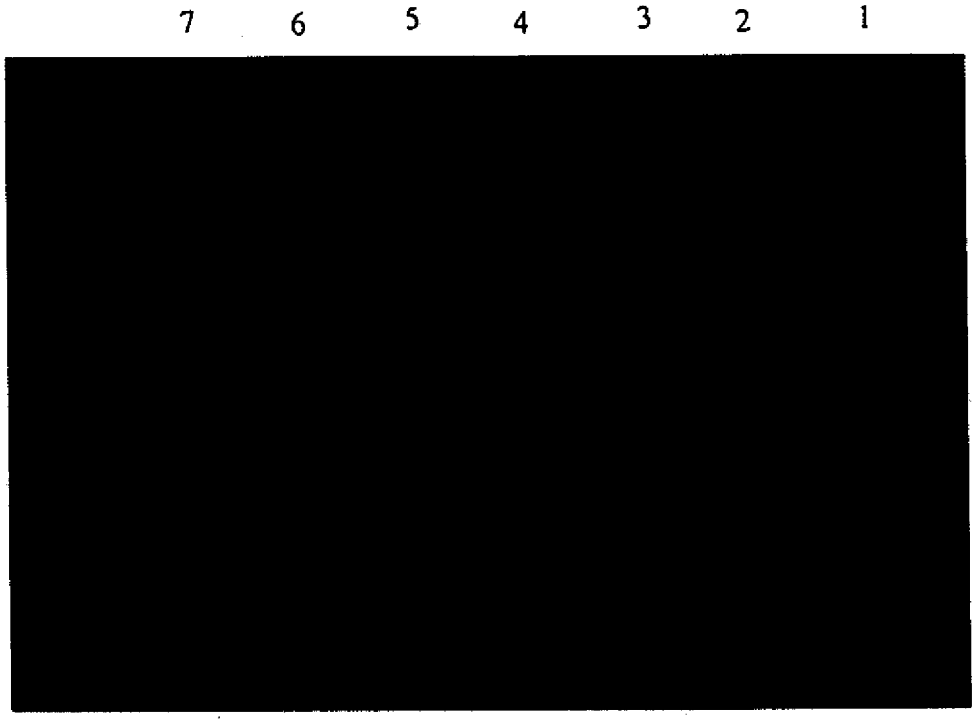
Fig. (9): Assessment of apoptosis by using DNA electrophoresis.



* **Lane 1-5**: Shows DNA electrophoresis of RSV infected HEP-2 cells. (Positive for apoptosis).
* **Lane 6:**Shows DNA electrophoresis of normal HEP-2 cells (negative for apoptosis).
*Lane 7: Shows DNA electrophoresis of RSV infected HEP-2 cells (negative for apoptosis).