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Anti-Herpes Effect and Mode of Action of Hesperidin as a Safe and Promising Alternative Antiviral

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ABSTRACT:

Genital ulcer disease is most commonly caused by types I and II of the herpes simplex virus (HSV). These viruses also cause infections of the genitalia. To evaluate the prevalence of HSV infection in Egypt and its treatment with hesperidin extract, this study was conducted. The antiviral and cytotoxic properties of hesperidin were thus assessed on 200 blood samples that were obtained for different types of analysis. Twenty (10%) of the 200 samples tested positive for HSV-I, and seven (3.5%) tested positive for HSV-II, according to our results. Furthermore, 6.75 log (10)/0.1 mL and 7.24 log (10)/0.1 mL, respectively, were the infectivity titers of HSV-I and HSV-II. HSV-I had overall values by PCR of 401–904 with a mean of 665.7 ± 163.5, while the HSV-II had overall values of 270–900 with a mean of 598.6 ± 243.4. There was no statistically significant difference between the HSV-I and HSV-II results by PCR, according to a statistical study between the two viruses (P = 0.517). Hesperidin's antiviral activity was seen to be 11% and 13.8% inhibition against HSV-I and HSV-II viruses, respectively, at a safe dosage of 312. 5 ug/mL and an IC_{50} of 682.22 ug/mL. Pre- and post-treatment analyses revealed hesperidin's mode of action, which was more effective against HSV-II than HSV-I (as revealed by the post-treatment approach). Finally, hesperidin's IC_{50} against HSV-II was 682.22 ug/mL, indicating a stronger viricidal impact.

Keywords: HSV, Herpes, Molecular assays, Antiviral, Hesperidin, Prevalence.

1-Introduction

The human herpes virus family includes the infectious alpha-herpes virus known as herpes simplex virus (HSV). It is a double-stranded DNA virus that produces a disease that lasts a lifetime after a single infection. HSV-I and HSV-II are the two distinct species that make up HSV [1, 2]. The herpes virus infects humans as well as a number of other hosts [1]. Herpes simplex virus (HSV) is a common human infection that can infect the genitalia (HSV-II) and the mucosal surfaces of the mouth, nose, and throat (HSV-I) [2]. The World Health Organization (WHO) estimates that 491 million people worldwide are impacted by HSV-II, while 3.7 billion are impacted by HSV-I, based on epidemiological data [3]. FDA-approved medications, such as acyclovir and its variants, are frequently used to treat HSV. These drugs work by blocking the enzyme HSV DNA polymerase, which is required for the replication process [4,5]. Despite these medications' usefulness in controlling outbreaks and reducing symptoms, drug resistance brought up by abuse has decreased treatment efficacy overall. Furthermore, antiviral treatments become more complex because of HSV's capacity to cause latent infections in host cells [6, 7].

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Scientists investigating the anti-HSV virus are constantly looking for safe, all-natural solutions to cure HSV infections. Research on the potential benefits of plant-derived flavonoids has been sparked by this quest [8-11]. Flavonoids are phenolic chemicals that are present in many different types of plants. Thus far, studies have been conducted on both the free and glycoside forms of numerous of these substances. However, a range of biological characteristics of flavonoids have been documented [12, 13], such as their chemopreventive, anti-inflammatory, anti-tumor, and anti-cancer actions. Flavone, a chemical compound with a C6 carbon skeleton based on aromatic rings, is also known as 2-phenylchromone or 2-phenyl benzopyrone (Fig. 1 A) [14]. Citrus fruits are rich in hesperidin (Hsd), a flavanone glycoside (a subclass of flavonoids) that has been shown to reduce HIV and herpes simplex virus type-II (HSV-II) [15]. Based on the findings of multiple research, hesperidin has been determined to be a potent antioxidant, anti-inflammatory, and anti-carcinogenic agent [16].

Hesperidin is an inexpensive, easily accessible drug [17]. The aglycone hesperetin (Fig. 1 B) is made up of the disaccharide rutinose and the similar aglycone hesperidin (Fig. 1 C) [18]. Hesperidin is thus ab-7-rutinoside hesperetin. The disaccharide unit ($C_{12}H_{22}O_{10}$), which can assume either of the two isomeric forms, rutinose or neohesperidose, is composed of one molecule of rhamnose and one molecule of glucose [19]. Studies have demonstrated the antiviral characteristics of many flavonoids, which may be helpful in the management of respiratory viral infections and other viruses [20]. While flavonoids have been known to have natural antiviral properties since the 1940s, attempts are currently being made to alter these molecules to increase their antiviral efficacy [21]. Citrus fruits, such as oranges, lemons, limes, grapefruits, mandarins, and others, have been found to contain the flavanone glycoside hesperidin ($C_{28}H_{34}O_{15}$) [22].

In citrus fruits, hesperidin is the most common flavonoid. This is especially true for sweet oranges, which can make up to 14% of the fruit's fresh weight when it is young and immature [23].

Moreover, it has been discovered that flavonoids, such as quercetin, apigenin, hesperidin, rutin, morin, and catechin, are efficient against eleven distinct viral kinds. The antiviral activity may be linked to non-glycosidic molecules [24]. Simultaneously, it appears that position 3 hydroxylation is necessary for the antiviral action of flavonols, which are purportedly more effective than flavones against the herpes simplex type-I virus; quercetin, kaempferol, and galangin were the order of significance [25]. Numerous pharmacological properties, such as anticancer, antihypertensive, antioxidant, antidiabetic, hepatoprotective, neuroprotective, wound healing, cardiovascular, anti-inflammatory, anti-obesity, hypoglycemic, lipid-lowering, and beneficial effects on bone and Alzheimer's disease, have also been demonstrated for hesperidin and its aglycone hesperetin [26, 27]. This study aimed to assess hesperidin's potential as a safe and effective alternative for herpes simplex virus types I and II, taking into consideration its varied biological activities.

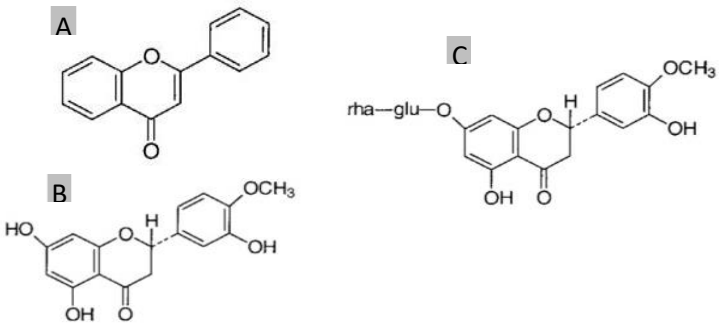

Figure1. A; flavone nucleus (2-phenylbenzopyrone), B; hesperetin (3, 5, 7-trihydroxy flavanone), and C; Hesperidin (hesperetin-7-rhamnoglucoside).

2. Materials and Methods

2.1. Sample collection

At the IBN SINA Lab, Beheira governorate, Egypt, 200 blood samples (Patients were not personally assisted in this study. Patients were informed and provided written consent for the collection of patient data and samples from the previously stated laboratory), were collected in total. In each EDTA tube blood sample, there was 5 mL of total blood. An aseptic centrifuge tube carried 2.5 mL of Ficoll-Hypaque density gradient separation solution when the 5 mL blood sample was coated onto the tube's side wall. After being left at room temperature for 20 minutes, the Ficoll-Hypaque blood was centrifuged at 2000 rpm. Four distinct layers form from the biological components of peripheral blood that separate during centrifugation. The uppermost layer was made up of platelets and plasma. A sterile vial containing carefully obtained plasma was kept at -80 °C until it was time to test it for antibodies and other biochemical processes. Within the layer between the plasma and Ficoll-Hypaque, peripheral blood mononuclear cells (PBMC) had a density of 1.77 g/mL. Above that were the erythrocytes [28].

2.2. Molecular Assay

2.2.1. Extraction of nucleic acid

RNA was extracted using the RTP® DNA/RNA Virus Mini Kit. 200 µL of the serum sample and 200 µL of dd H_2O were added to the extraction tubes that were provided. If the volume of the sample was less than 200 µL, 400 µL of dd H_2O was added, and it was heated in a thermomixer for 10 minutes at 95 °C before it was incubated for 15 minutes at 65 °C. For optimal binding conditions, 400 µL of the binding solution was added and thoroughly mixed up and down with a pipette. After being moved to the RTA Spin filter, the sample was centrifuged for two minutes at 11.000 x *g* (11.000 rpm) and allowed to incubate for one minute. After discarding the flow-through and RTA receiver tube, the RTA Spin filter was placed in a brand-new RTA receiver tube. After centrifuging the RTA Spin filter for a minute at 11.000 x *g* (11.000 rpm) with 500 µL of wash buffer R1, the RTA receiver tube and the flow-through were disposed of. A new RTA receiver tube was filled with the RTA Spin filter. After being pipetted-washed with 700 µL of wash buffer R2, the RTA Spin filter was centrifuged for one minute at 11.000 x *g* (11.000 rpm), and the flow-through was disposed of. The filter was then placed in a new RTA receiver tube. We threw off the RTA receiver after centrifuging four more times at high speed to eliminate any remaining ethanol. Pipetting directly onto the membrane, 60 µL of the 65 °C-preheated elution buffer R was added to a 1.5 mL Rnase-free elution tube containing the RTA Spin filter. After three minutes of incubation, the tube was centrifuged at 11.000 x g (11.000 rpm) for one minute. The RTA Spin filter was eventually discarded, and the viral DNA/RNA that had been eluted was put on ice [29].

2.2.2. Nucleic acid purification

Nucleic acid extraction has to be done with extraction kits that are easily obtained from the market, following the protocols for the particular clinical material extraction. What the manufacturer recommends is the extraction kit below: Utilizing the croBEE NA16 Nucleic Acid Extraction System, the GeneProof PathogenFree DNA Isolation Kit is available. The IS needs to be injected right away into the sample at the beginning of the isolation process to guarantee that 0.1 µl of the IS is present in 1 µl of the final elution volume when using the ISEX versions of the PCR kits [29]: 25 (µL) to 200 (µL) elution volumes interior Standard: 2.5, 5, 10, 20, µL.

2.2.3. PCR amplification

In each of the three areas where the primers matched the 721-bp fragment, they were amplified, as indicated by Tables 1 and 2. iNtron, Korea supplied 50 µl of PCR master mix that included 5.0 U of Taq polymerase, 0.5 µM primers, 0.22 mM deoxynucleoside triphosphate, and 1.6 mM MgC_{l2}. Three minutes of heating at 94°C, one minute of denaturing at 94 °C, 45 seconds of annealing at 70 °C, and one minute of elongation at 70 °C with a 3-second cycle extension comprised the 35 cycles of the procedure in a Bio-Rad DNA thermal cycler. An agarose gel was used to separate the PCR findings, and then a MEGA total fragment DNA purification kit was used to recover the relevant DNA bands from the gel.

Table 1: Primers used for detection of HSV- I

Primer	Nucleotide positions	Sequence
1	4046–4068	5′TGTTTCAACAGAAATGACCGCCC3′
2	4421–4402	5′CTCAAGATGTTCGCCGTCCC3′
3	4335–4352	5′ACGCCCGACCACACACCC3′
4	4667–4648	5′TATGTTGAGGCGTCGGAACC3′
5	4563–4580	5′AGTCGCCCGAAGACACCC3′
6	4892–4875	5′CCGCATGTGGGCTCTCCC3′

Table 2: Primers used for detection of HSV- II

Primer	Nucleotide positions	Sequence
F	24669–24680	5′-ACCCGATCTACGACGAAGTG3′
R		3’-CATCGCGTCACACCAGAC-5’

2.3. Antiviral and Cytotoxicity Measurements

Hesperidin (orange peel extract) and interferon (vacsera, employed as a positive control) were used in an antiviral test against herpes simplex virus types I and II on Vero cells. To aid in the proliferation of Vero cells, 0.1% antibiotic/antimycotic solution and 10% fetal bovine serum were added to DMEM media. The fetal bovine serum, DMEM medium, antibiotic and antimycotic solution, and trypsin-EDTA were provided by Gibco BRL (Grand Island, NY, USA). Cytopathic inhibitory effect (CPE), which was recently identified, was assessed using the crystal violet technique to assess antiviral activity and cytotoxicity assays. A day before infection, Vero cells were, in essence, planted at a density of $2x10^4$ cells/well into a 96-well culture plate. Following the removal of the media the next day, phosphate-buffered saline was used to wash the cells. The crystal violet method, which displayed CPE and allowed the percentage of cell viability to be determined, was used to analyze the ability of Herpes simplex virus types I and II to infect cells. $CCID_{50}$ (1.0×10^4) of viral stock was added to 0.1 mL of diluted herpes virus solution for mammalian cells. The purpose of choosing this dose was to guarantee that, two days after infection, the appropriate CPEs would form.

To give the cells medication, 0.01 mL of medium was mixed with the necessary amount of the drug. A ten-fold diluted concentration range of 0.1-100 µg/mL was used to evaluate each test sample's antiviral efficacy. Both viral control (virus-infected, untreated cells) and cell control (untreated, non-infected cells) are at play. For 72 hours, culture plates were incubated at 37 °C with 5% CO_2. The evolution of cytopathic impact was studied using light microscopy. The cell monolayers were washed with PBS and then fixed and stained with a 0.03% crystal violet solution in 2% ethanol and 10% formalin. Spectrophotometry was used to measure each well's optical density at 570/630 nm after washing and drying.

The percentage of antiviral activities of the compounds tested, as reported by Pauwels et al. [30],

was determined by applying the following equation: antiviral activity= [(mean optical density of virus controls - mean optical density of cell controls)/ (optical density of test - mean optical density of virus controls)] Ó100%. It was from these results that the 50% CPE inhibitory dose (IC_{50}) was computed. Before this experiment, a 96-well culture plate was seeded with cells at a density of $2x10^4$ cells/well to examine the cytotoxicity. The next day, the cells were treated with the culture medium, which included samples that had been diluted step-by-step. After 72 hours, the culture medium was removed, and PBS was applied to the cells. The following steps were carried out using the same protocols as described above for the antiviral activity assay. The 50% cytotoxic concentration (CC_{50}) and 50% inhibitory concentration (IC_{50}) were estimated using GraphPad PRISM software (Graph-Pad Software, San Diego, USA) (31).

$$50\%\ \text{endpoint}\ (\text{CCID}_{50}) = \frac{\%CPE > 50 - 50const \times log}{\%CPE > 50\% - \%CPE < 50\%}$$

2.4. Statistical analysis

Data collected were reviewed tabulated and analyzed utilizing SPSS (statistical package for social services). Program version 16 the level of significance was taken at $p<0.05$ [32].

3. Results

3.1. Prevalence of HSV in collected samples

Only twenty-seven samples (27/200, 13.5%) tested positive for the herpes simplex virus; twenty samples tested positive for HSV-I (20/200, 10%; twenty/27, 74.1%); and seven samples tested positive for HSV-II (7/200, 3.5%; 7/27, 25.9%). According to Table 3, Figs. 4&5, the statistical analysis revealed that HSV type-I was considerably more common than HSV type-II in the study group at *P* value = 0.01.

Age: In type-I HSV, there were 13 cases (13/20, 65%) and 7 cases (adults, 35%), while in type-II HSV, there were 7 cases (adults, 100%). According to the statistical analysis, type-II HSV was more common in adults (*P* = 0.001 and 0.021, respectively), although type-I HSV was significantly more common in the pediatric age range (Table 4, Fig. 6).

In the HSV-I group, the overall age ranged between 3-50 years with a mean of 17.8±16.1 years while in the HSV-II group, the overall age ranged between 18-42 years with a mean of 30.3±8.5 years. The statistical analysis (*P* = 0.018) revealed that the mean age of HSV-I-affected cases was significantly lower in both groups (Table 5, Fig. 7). Pediatric patients in the HSV-I group had an average age of 7.54 ± 4.2 years, but adult patients in the HSV-I group had an average age of 18–50 years, with a mean of 36.9 ± 11.8, and adult patients in the HSV-II group had an average age of 30.3 ± 8.5 years. According to the statistical analysis, there was no statistically significant difference (*P* = 0.258) between people infected with HSV-I and HSV-II (Table 5, Fig. 7).

Sex: Of the type-I HSV cases, six (17/20, 30%) were female and fourteen (14/20, 70%) were male. Two cases (2/7, 28.6%) and five instances (5/7, 71.4%) of type-II HSV were male. A statistical study revealed that type-I HSV considerably affected males (*P* = 0.021) and type-II HSV significantly affected females (*P* = 0.022); additionally, type-II HSV affected females more than type-I HSV (*P* = 0.021). Six females (6/20, 30%) and fourteen males (14/20, 70%) were found in type-I HSV. The statistical analysis showed that in type-I HSV, males were generally affected more frequently than females in all age groups (pediatrics and adults) (*P* = 0.021, 0.023, and 0.01 respectively). Of those patients, thirteen belonged to the pediatric age group, eight of them (8/13, 61.5%), and five (5/13, 38.5%) were females. Seven cases belonged to the adult age group. Six of them (6/7, 85.7%) were males, and only one was female (1/7, 14.3%).

Table 3: Distribution of collected samples

Variable	No.	Percent (%)	*P*
Free samples	173	86.5%	
Positively detected samples:	27	13.5%	
Herpes Simplex type-I	20	74.1%	0.01
Herpes Simplex type-II	7	25.9%	(S)

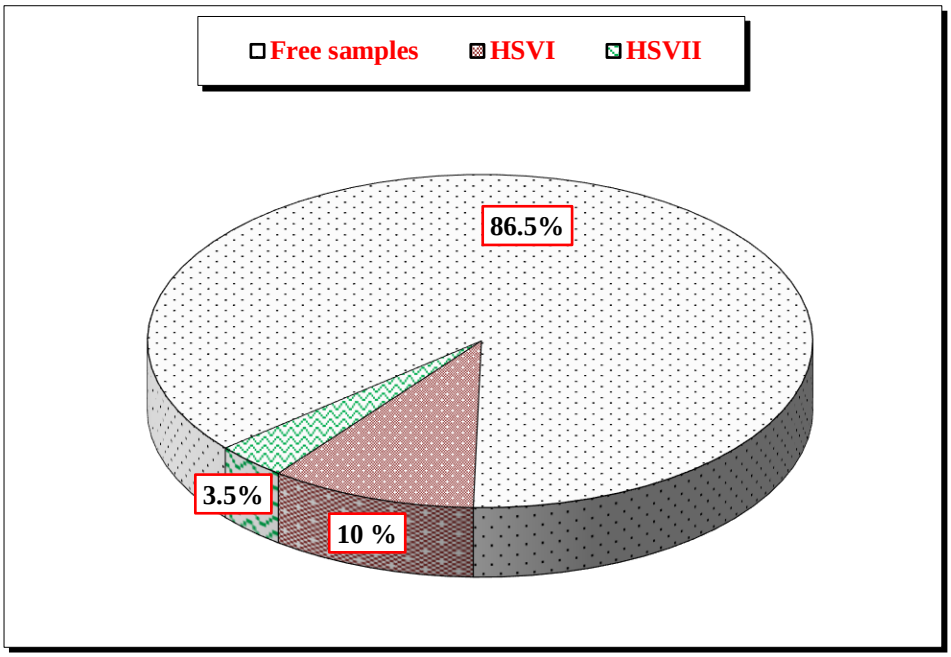

Fig. 4: Distribution of infection in the studied group.

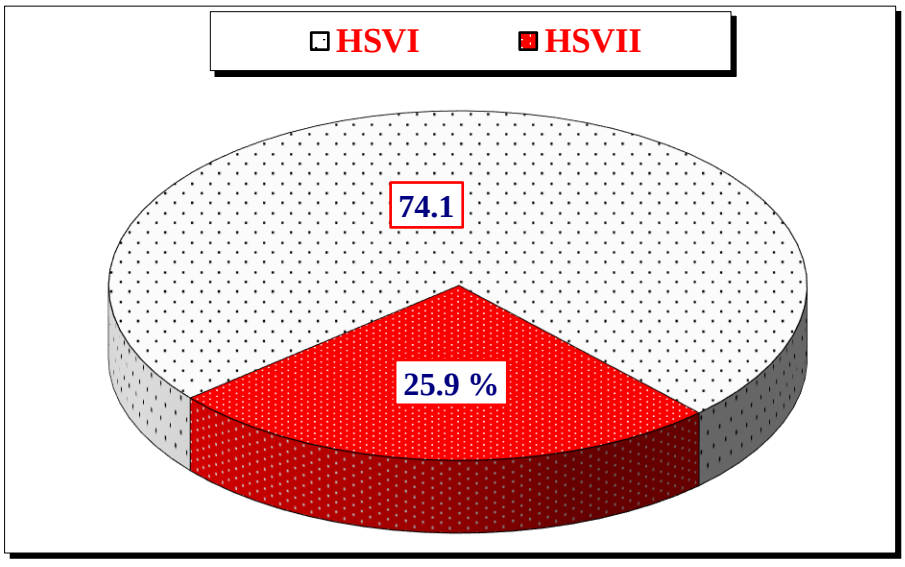

Fig. 5: Distribution of positive samples of HSV.

Table 4: Distribution of age groups in the studied patients.

Variable	Adult (No. /%)	Pediatrics (No. /%)	P
HSVI	7 (35%)	13 (65%)	0.021 (S)
HSVII	7 (100%)	0 (0.0%)	0.001 (S)
P	0.01 (S)	0.001 (S)	

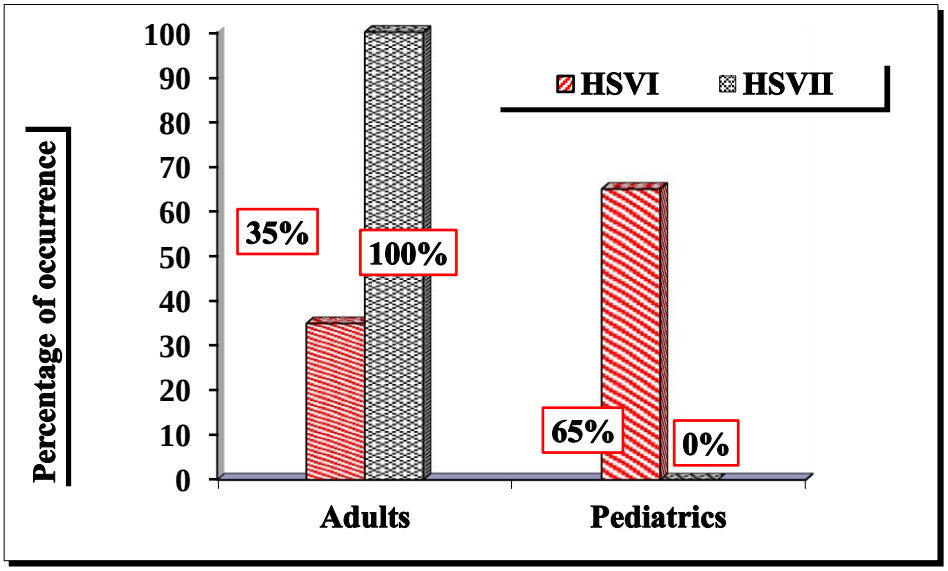

Fig. 6: Distribution of age groups in the studied patients.

Table 5: The mean age in the studied groups.

Variable	Adult	Pediatrics	Overall
HSVI			
Range	18-50	3-18	3-50
Mean±S.D	36.9±11.8	7.54±4.2	17.8±16.1
HSVII			
Range	18-42	0-0	18-42
Mean±S.D	30.3±8.5	0-0	30.3±8.5
P	0.258		0.018 (S)

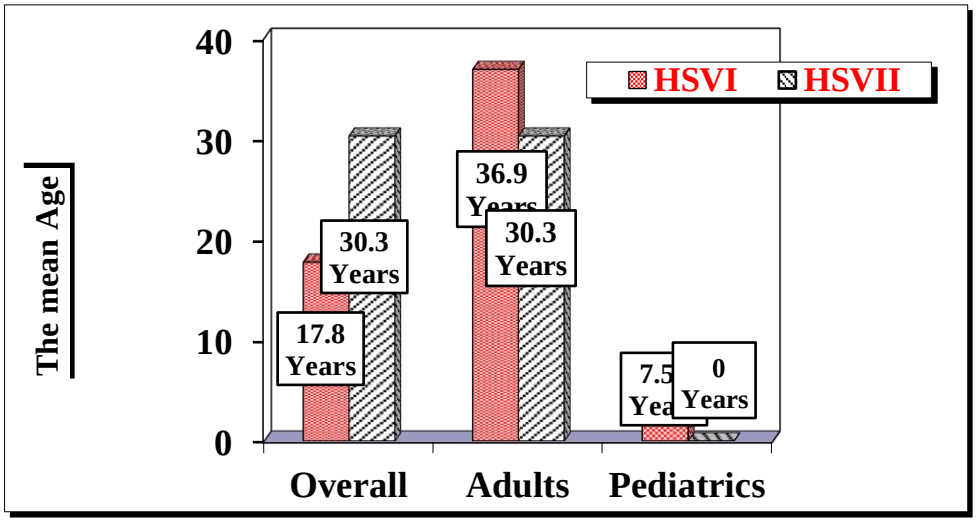

Fig. 7: The mean age in the studied groups.

Table 6: Sex distribution in the studied patients.

Variable	Male (No./%)	Female (No./%)	*P*
HSVI	14 (70%)	6 (30%)	0.021 (S)
HSVII	2 (28.6%)	5 (71.4%)	0.022 (S)
P	0.023 (S)	0.021 (S)	

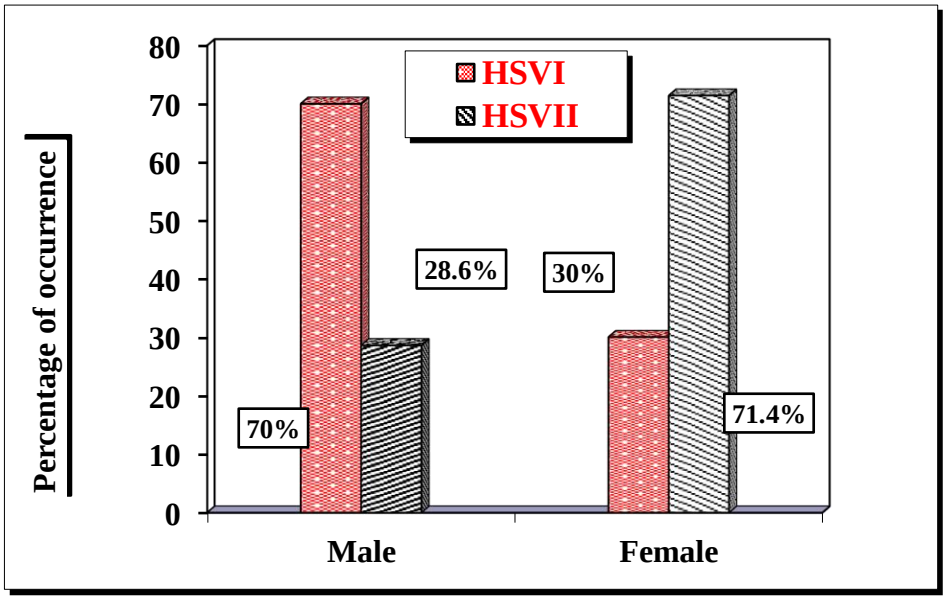

Fig.8: Sex distribution in the studied patients.

Table 7: Sex distribution in HSVI-studied patients.

Variable	Male (No./%)	Female (No./%)	*P*
Overall	14 (70%)	6 (30%)	0.021 (S)
Pediatrics	8 (61.5%)	5 (38.5%)	0.023 (S)
Adults	6 (85.7%)	1 (14.3%)	

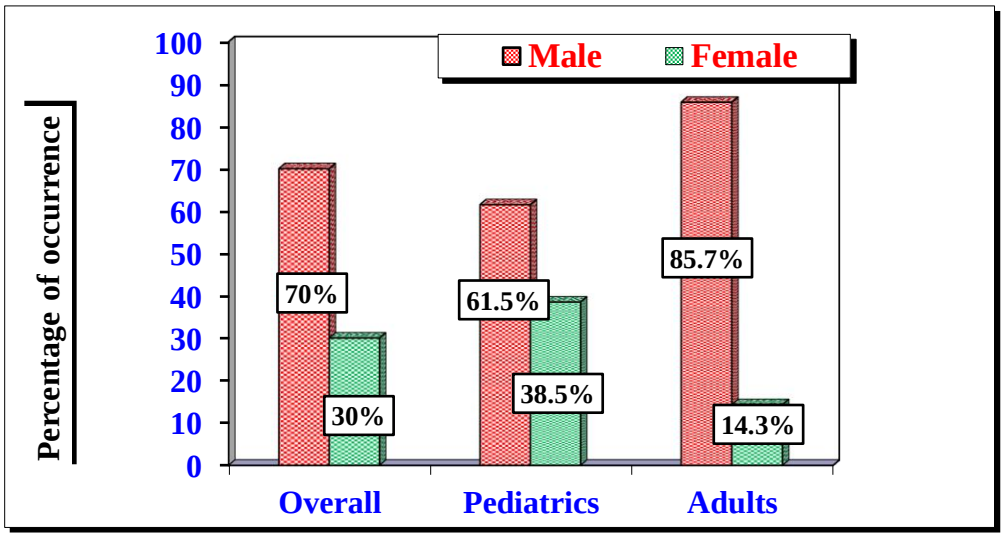

Fig. 9: Sex distribution in HSVI-studied patients.

3.2. Infectivity titer of HSV-I

One plate of Vero cells was used as a control to examine the infectivity of HSV-I on Vero cells; in this case, the cells multiplied without obtaining an HSV-I inoculation (Fig. 10 A). After applying a viral treatment, the Vero cells were left to incubate on a separate plate for a whole day (Fig. 10 B). The aggregation that happens when cultured cells are compared to untreated control cells in Figures (Fig. 10 A) and (Fig. 10 B) illustrates the cytopathic effect of HSV-I on Vero cells. 6.75 log (10)/0.1 mL was the measured and observed HSV-I infectivity titer.

3.3. Infectivity titer for HSV-II

For 24 hours at 37 °C, untreated Vero cells served as the control group, and HSV-II was not added (Fig.11 A). A whole day was spent developing and cultivating more HSV-II-infected cells at 37 °C. There was a minor aggregation of these cells during incubation (Fig. 11 B), indicating that HSV-II may have a cytopathic effect on Vero cells. About 7.24 log (10)/0.1 mL was the HSV-II infectivity titer seen and assessed.

3.4. Cytotoxic effect of hesperidin

As shown in Figure (12), the MTT test was performed to ascertain the safe concentration of hesperidin on Vero cells when the percentage of viable cells was blotted against hesperidin (ug/mL). 91.55% of the cells were viable at the safe dose of 312. 5 ug/mL.

3.5. IC_{50} for hesperidin

By calculating the IC_{50} with the use of the Masterplex-2010 program, the inhibitory concentration of hesperidin was evaluated. Figure (13) illustrates that hesperidin's IC_{50} was 682.22 ug/mL.

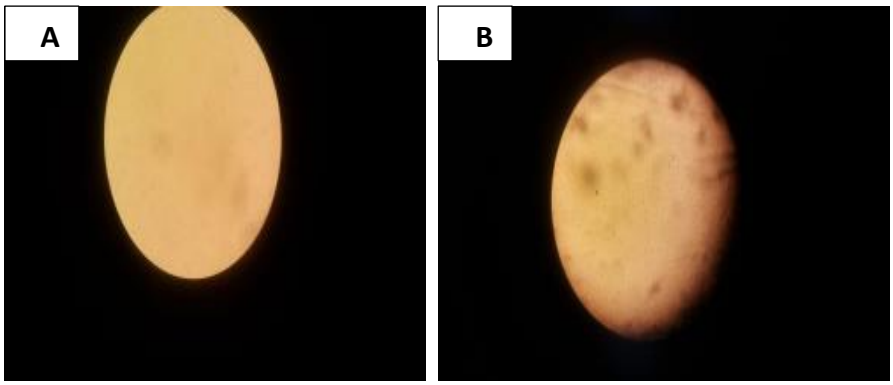

Fig. 10: A; Control Vero cells, B; cytopathic effect of HSV-I after 24 h post infection.

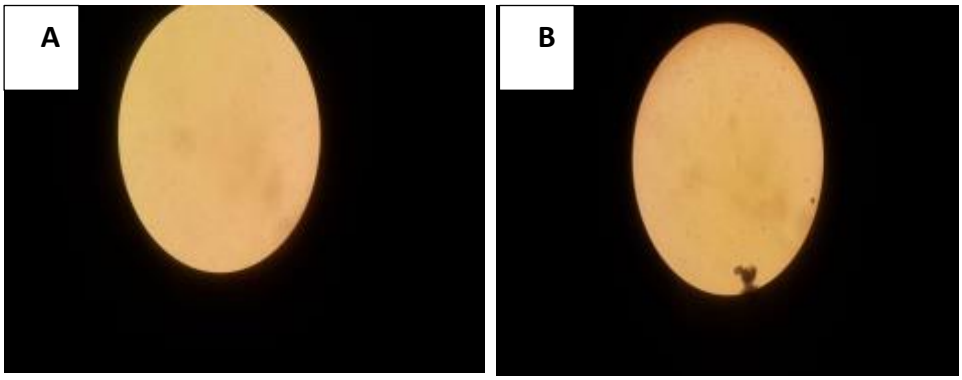

Fig. 11: A; Control Vero cells, B; cytopathic effect of HSV-II after 24 h post-infection

3.4. Cytotoxic effect of hesperidin

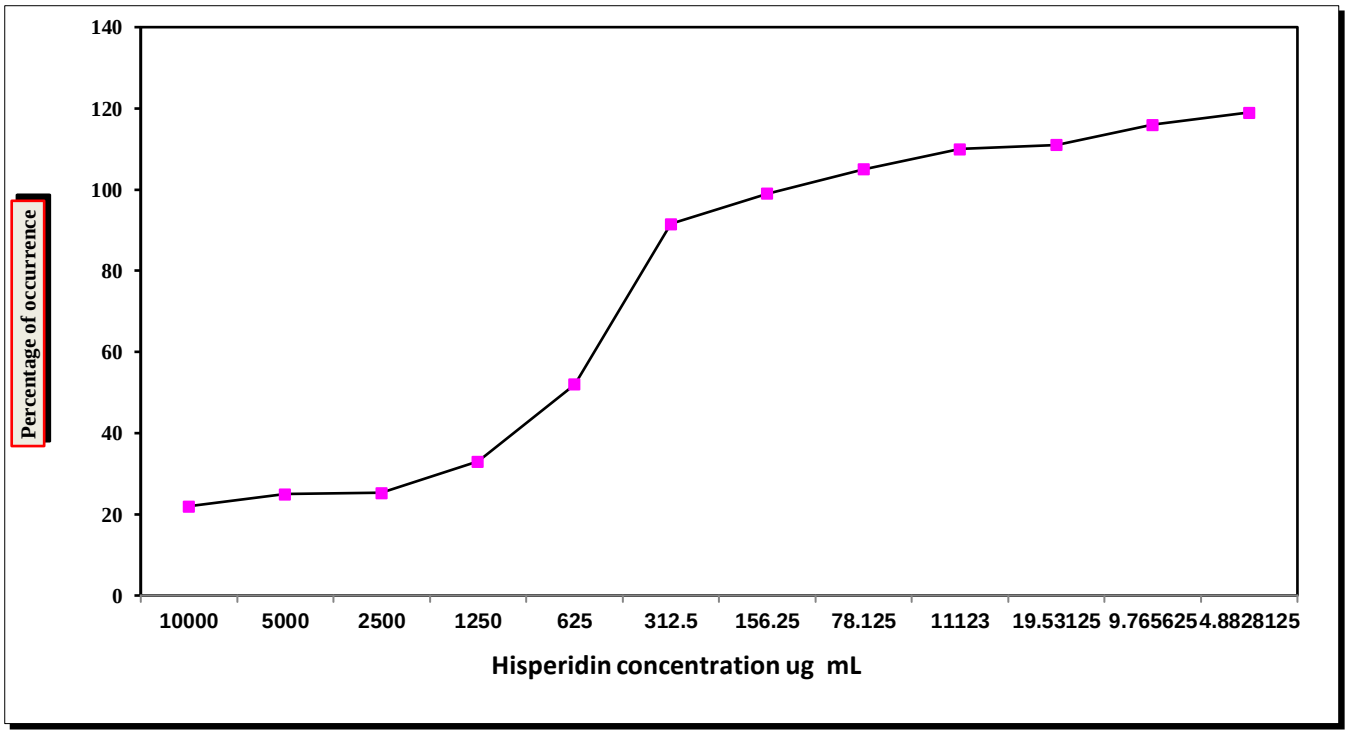

Fig. 12: Evaluation of Vero cell viability using an MTT assay

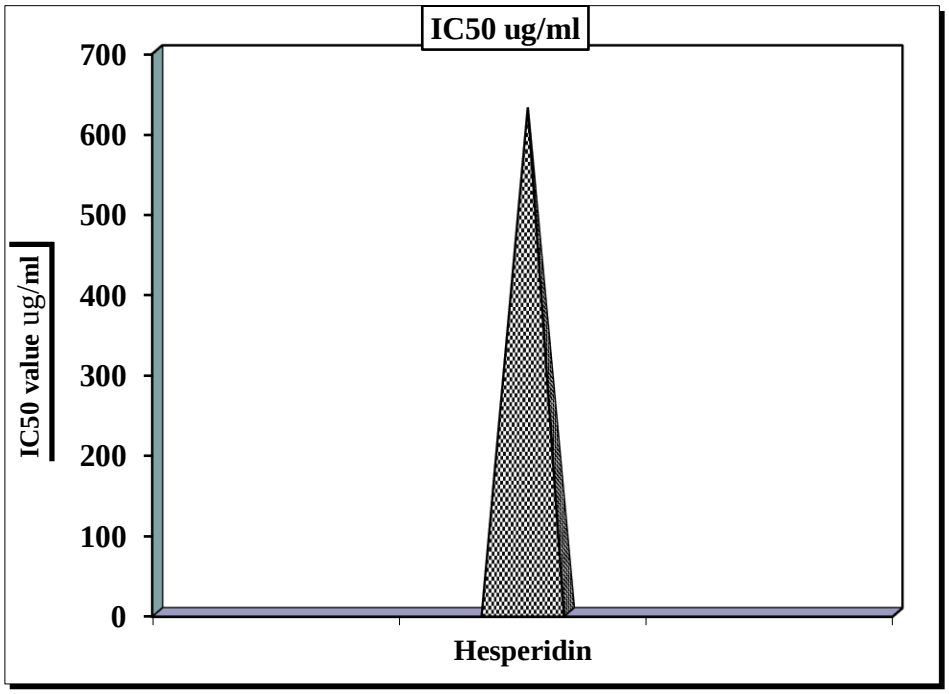

Fig. 13: Half maximal inhibitory concentration (IC_{50}) of hesperidin

3.6. Direct antiviral activity test of hesperidin

After virus titration, hesperidin's direct antiviral potential against the test viruses was calculated using the previously determined safe dose of 312.5 ug/mL, and its effects were recorded. Figure (14) shows that the HSV-I virus titer was 6.75 log (10)/0.1 mL before therapy; 48 hours later, the titer decreased to 6 log (10)/0.1 mL, suggesting 11% inhibition. HSV-II showed a 13.8% inhibition as evidenced by the titer dropping from 7.24 log (10)/0.1 mL to 6.24 log (10)/1 mL. It was demonstrated that hesperidin had a more potent antiviral effect against HSV-II than HSV-I.

3.7. Mode of action test for hesperidin (pre-treatment)

By giving hesperidin to Vero cells for two hours and then discarding it after the viruses were added separately, the antiviral efficacy of the substance was evaluated. The HSV-I titer decreased from 6.75 log (10)/0.1 mL to 6.5 log (10)/0.1 mL in Figure (15), demonstrating a 3.7% inhibition. Conversely, when HSV-II's titer decreased from 7.24 log (10)/0.1 mL to 5.75 log (10)/0.1 mL, it demonstrated a 20.6% inhibition. Hesperidin showed greater activity against HSV-II than HSV-I.

3.8. Mode of action test of hesperidin (post-treatment)

By giving hesperidin to Vero cells for two hours and then discarding it after the viruses were added separately, the antiviral efficacy of the substance was evaluated. The HSV-I titer decreased from 6.75 log (10)/0.1 mL to 6.5 log (10)/0.1 mL in Figure (15), demonstrating a 3.7% inhibition. Conversely, when HSV-II's titer decreased from 7.24 log (10)/0.1 mL to 5.75 log (10)/0.1 mL, it demonstrated a 20.6% inhibition. Hesperidin showed greater activity against HSV-II than HSV-I.

3.9. Antiviral activity of Interferon (INF)

Defining INF's antiviral effect regarding Vero cells. Figure 17 illustrates that before the injection of IFN, the infectivity titer for HSV-I was 6.75 log (10)/0.1 mL. The titer was 3.24 log (10)/0.1 mL after INF was added, suggesting an activity against HSV-I. But the HSV-II titer decreased from 7.24 log (10)/0.1 mL to 3.75 log (10)/0.1 mL after INF was added. There was an overall 52% reduction in the HSV-I titer and approximately 48% reduction in the HSV-II titer.

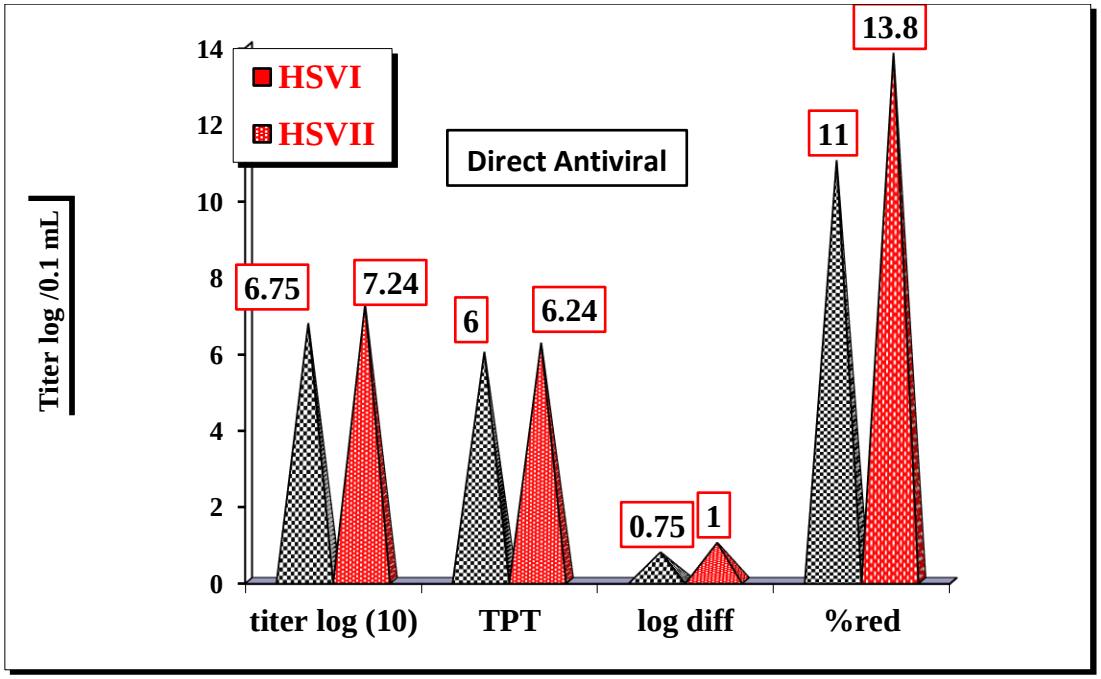

Fig.14: Direct Antiviral activity of hesperidin against HSV-I and HSV-II. TPT = Titer post-treatment, %red = reduction percentage.

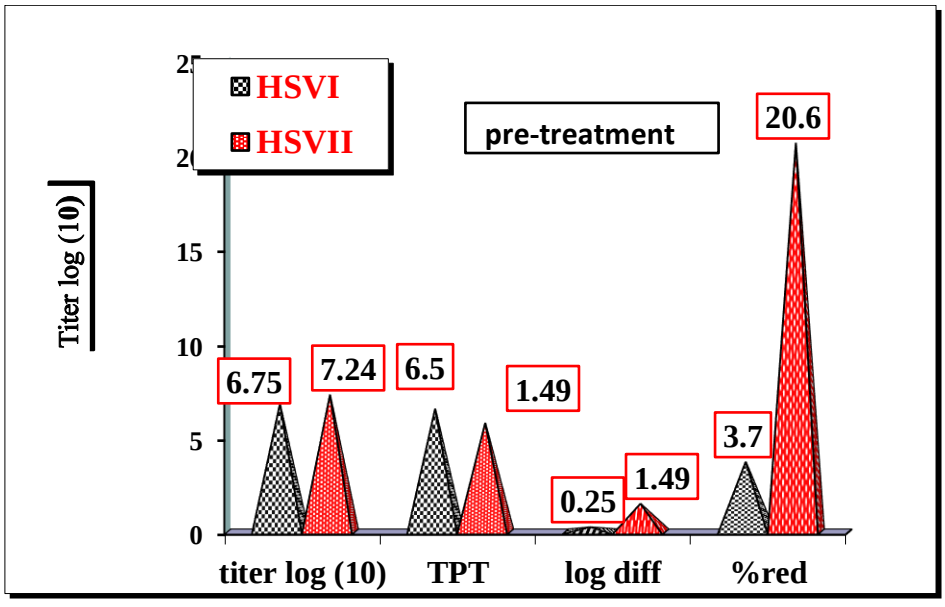

Fig. 15: Mode of action (pre-treatment) activity of hesperidin against HSV-I and HSV-II. TPT = Titer post-treatment, %red = reduction percentage.

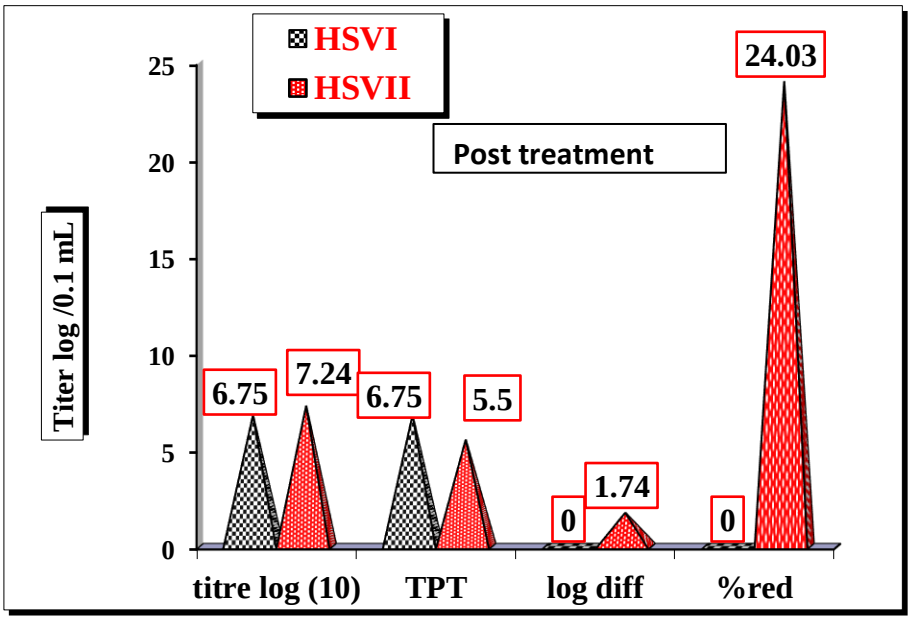

Fig. 16: Mode of action (post-treatment) activity of hesperidin against HSV-I and HSV-II. TPT = titer post-treatment, %red = reduction percentage.

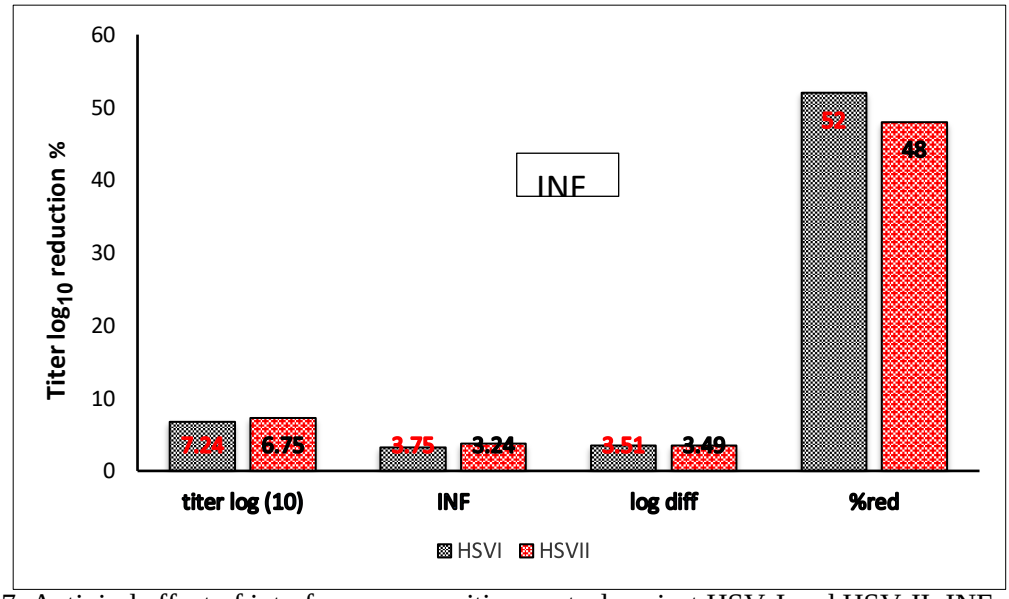

Fig. 17: Antiviral effect of interferon as a positive control against HSV-I and HSV-II. INF = interferon,% red = reduction percentage.

4. Discussion

Herpes simplex virus type-1 (HSV-I) is a highly contagious illness that can last a lifetime and is quickly acquired in childhood [33-35]. 2012 is expected to see 118 million new infections worldwide, a figure far higher than the 19 million new cases of HSV-II infection that were reported in 2011 [36]. Worldwide, HSV-I infection is widespread and never totally goes away [37- 39]. The statistical analysis of the present study's data revealed that HSV type-I was more common than HSV type-II in the group under investigation, that type-I HSV was more common in children than type-II HSV in adults, and that type-I HSV was more common in men than type-II HSV in women (P = 0.021 and 0.022, respectively). Men were also more

impacted by type-I HSV than type-II HSV (P = 0.023), while women were more affected by type-II HSV than type-I HSV (P = 0.021).

HSV-II is the primary virus responsible for the majority of cases of genital herpes [40], according to According to Hu et al. [40], HSV type-I is more prevalent in youngsters and often results in oral infections, while HSV-II is the primary virus responsible for the majority of genital herpes cases [40]. According to Ayoub et al. [41], 25% of acquisitions in the 15–49 age range will be genital, meaning that young individuals will bear the brunt of the HSV-I infection, which is expected to be more common in those under the age of 15.

A possible consequence of the increasing frequency of genital HSV-I infections in women of reproductive age is an increase in the prevalence of neonatal herpes, a very fatal condition with a high risk of death [42, 43]. Roughly 16% of the world's population between the ages of 15 and 49 was projected to have HSV-II in 2003, or 536 million individuals. Being a chronic ailment, infection is expected to become more common with age, and the results support this idea. Women had a higher prevalence than men, according to previous research [44].

Hesperidin has been identified as a powerful anti-inflammatory, antioxidant, and anti-carcinogenic agent based on the findings of multiple in vivo and in vitro investigations [6, 45]. It has been demonstrated that hesperidin is more concentrated in the membranes, albedo, membranes, and pith of citrus species than in the seeds and juice vesicles [46, 47]. The assessment procedure was used to calculate the infectivity titer of HSV-I and HSV-II using the cell culture infectious titer calculation. Next, assays for cell growth and cytotoxicity were used to evaluate the antiviral and mechanism of action activities of hesperidin. In a study published in 48, Paredes et al. evaluated the impact of naringenin, hesperetin, and its glycoside (hesperidin) form on the in vitro replication of the Sindbis neurovirulent strain (NSV). All of the flavanones were declared non-cytotoxic after testing on Baby Hamster Cells 21 Clone 15 (BHK-21). Using the MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyl -tetrazolium bromide) colorimetric assay and the plaque reduction assay, the antiviral action was evaluated. NSV infection was inhibited by both hesperetin and naringenin [48, 49]. Two of their glycosides, hesperidin, and naringin, did not inhibit the virus. Hesperidin, however, had a modest antiviral impact on HSV-II in our investigation when treated with a mode of action (post-treatment) of 24.03%; the virus titer log difference was 1.74 log 10/0.1 mL.

Our analysis revealed that the safety concentration of hesperidin in MTT-assayed Vero cells was 312.5 ug/mL. In a recent work by Parvez et al. [49], the maximal dose of hesperidin used on HBV-reported cells, HepG 2.2.15 (a human hepatoblastoma line derived from HepG2), was 50 ug/mL. This implies that there was no cytotoxic effect on the cells, which was confirmed by microscopy and the MTT assay. Many factors, such as the hesperidin's purity after filtering in the two investigations, the hesperidin's preservation conditions, and the cell proliferation assay that was performed, could account for this disagreement between the two computed concentrations [50, 51].

Additionally, on day five after therapy, Parvez et al. [49] assessed the compounds' antiviral activity, and the findings were as follows: Quercetin is present in 70% of the sample, followed by Psoralen (67%), Menisdaurin (64%), Baccatin III (71%), Embelin (65.5%), Azadirachtin (62%), Lupeol (52.5%), Rutin (47, 5%), Bsitosterol (43%), and Hesperidin (41%). Every percentage has a corresponding order. Six of the compounds had strong anti-HBV activity: rutin, bsitosterol, lupeol, and hesperidin; the other four only showed mild resistance. Psoralen, quercitin, baccatin III, embelin, menisdaurin, and azadirachtin were the other five. However, a 3.7% drop in the HSV-I titer log indicated that hesperidin had only a weak antiviral impact in our investigation [50, 51]. Following the direct application of hesperidin, the endpoint assay yielded a titer log

difference of 0.75 log/0.1 mL for HSV-I, suggesting an antiviral response that was below average. Yet in research by Castrillo et al. [52], they investigated the antiviral effects of the non-cytotoxic flavanones hesperidin and its glycoside derivatives (hesperidin) on the 17D strain of yellow fever virus (YFV17D) reproduction using a plaque suppression experiment. Even when the concentration was maximum, the glycosylated form of hesperidin had no antiviral effect. The highest concentration of hesperidin was applied to the opposite band, fully eliminating the infectious titer and plaque size.

The virus had already infected 80% of the cells after two hours of infection; however, hesperidin prevented this entry, bringing the infection down to 55.2% [22, 52]. It was discovered that interferon outperformed hesperidin in terms of antiviral activity against both viruses. Compared to a 52% reduction with interferon, hesperidin only showed an 11% reduction in the HSV-I titer. Interferon therapy resulted in titer inhibition of around 48% for HSV-II, whereas hesperidin showed titer inhibition of about 13.8% [53, 54]. To prevent infections, interferon maintains barrier integrity at epithelial surfaces and fosters antiviral immunity [53, 54]. The most powerful antiviral transcriptional program of interferon targets epithelial cells, while leukocytes, including neutrophils and dendritic cells (DCs), are the source of interferon's ability to elicit a protective response.

5. Conclusion and Recommendation

The search for safe, natural treatments for HSV infections is a constant focus for researchers studying anti-HSV therapy. A thorough search for workable natural HSV control alternatives is made possible by the study's findings. With an IC_{50} of 682. 22 ug/mL, hesperidin showed higher viricidal efficacy against HSV-II than HSV-I at both pre-and post-assay sessions. Roughly 312. 5 ug/mL was the acceptable concentration. HSV-I and HSV-II were found to be more susceptible to the antiviral action of interferon. In contrast to interferon, which lowered HSV-I titer by 52%, hesperidin reduced it by 11%. In contrast to interferon treatment, hesperidin demonstrated titer inhibition of roughly 48% against HSV-II. It is suggested that hesperidin shows promise in managing HSV, thus further research should be carried out to boost its activity .

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