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# *In Vitro* Antidiabetic Effect of Neohesperidin

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## ABSTRACT

**Objective:** The present study was performed to determine *in vitro* antidiabetic effect of neohesperidin. To evaluate inhibitory effect of neohesperidin on α-amylase and α-glucosidase diabetes causing enzyme.

**Methods and Materials:** *Invitro* carbohydrate metabolizing enzyme based inhibitory methods were used to determine antidiabetic effect of neohesperidin. Alpha (α)-amylase inhibitory assay was performed using different sources i.e. wheat alpha (α)-amylase enzyme, salivary alpha (α)-amylase and fungal alpha (α)-amylase assay. Alpha (α)-glucosidase inhibitory assay was performed using alpha (α)-glucosidase *(B. stearothermophil)*, alpha (α)-glucosidase rat intestine and alpha (α)-glucosidase from baker's yeast. Sucrase inhibitory assay from rat small intestine.

**Result:** Neohesperidin possess a potent anti-diabetic by significantly inhibiting alpha amylase activity.

**Conclusion:** It was concluded that enzyme inhibitory activity of neohesperidin shown a significantly higher inhibitory activity on alpha-amylase in comparision to alpha-glucosidase & Sucrase enzymes.

**Keywords:** Neohespiridin, acarbose, alpha-amylase, alpha-glucosidase

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## INTRODUCTION:

Neohesperidin (hesperetin-7-neohesperidoside) are flavanones glycosides unique to Citrus and specific citrus cultivars belongs to family rutaceae.[1,2]They are weak-polar molecules with bitter taste and their content or ratio can be used as citrus taxonomic markers [3,4] or indicators for identification of authentic orange juice *(Citrus sinensis)*.[5] They share the same neohesperidose (2-O-a-Lrhamnopyranosyl-b-D-glucopyranose), which determines their bitterness, as naturally occurred flavonoids; they are known dietary antioxidants that have diverse biological activities. [6]It have been reported that neohesperidin possess antioxidant[7, 8, 9],antidiabetic [10-13], hypolipidemic effects [14-17], protection against cardiovascular diseases [18], anti-inflammatory[19-21], anti-arthritic activity, anti-carcinogenic [22] and antimicrobial property [23, 24].Natural amylase and α-glucosidase inhibitors from food-grade plant sources offer an attractive strategy to control post-prandial hyperglycaemia. Natural inhibitors from plants, which have been shown to have a low inhibitory effect against α-amylase activity and a strong inhibition activity against α-glucosidase, can be used as an effective therapy for postprandial hyper glycaemia with minimal side effects. [25]

## MATERIALS AND METHODS

### Chemicals and reagents:

Alpha-glucosidase enzyme, Neohesperidin, 4-Nitrophenyl α-D-glucopyranoside, Calcium acetate (Sigma - Aldrich, USA); Potassium dihydrogen orthophosphate, Sodium hydroxide pellets, Sucrose, Maleic acid (Loba Chemie PVT LTD Mumbai India); Sodium potassium tartarate, Starch soluble, Yeast powder(Fizmerk India Chemicals); petroleum ether (Samar chemicals, India); Acarbose (Bayer pharmaceuticals Pvt. Ltd, Baddi); Sodium dihydrogen orthophosphate (Molychem, Mumbai) and Sodium carbonate anhydrous (Merk Life Science Private Limited, Mumbai) etc.

### Chemistry of neohesperidine:

Its IUPAC name is methoxyflavanone-7-beta-neohesperidoside 3,5,7,4 tetrahydroxy, it is also known as hesperetin 7-O-neohesperidoside. Structurally it belongs to the class of flavanones. Its molecular formula is $C_{28}H_{34}O_{15}$ and molecular

weight is 610.5606g/mol. It is stored in cold condition i.e. 2-8 °C.

**Enzyme inhibitory activities**

***Wheat alpha amylase inhibitory activity:***

500 gm of malted whole wheat flour was added slowly with stirring to 1 liter of 0.2% calcium acetate solution at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was then centrifuged at 40ºC at 12000 rpm for 10 minutes. The resultant clear brown supernatant was stored at 2°C to 3°C prior to heat treatment. Then inactivates β-amylase by heat treatment, since beta amylase interferes with the enzymatic determination of alpha amylase it was inactivated by heating the extract at 70°C for 15 minutes. Alpha amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to pH 6.6 with cold 4% ammonium hydroxide. Heat treatment was carried out at 85°C to 90°C and other at 72°C to 74°C using a water bath with continuous stirring. The extract was then cooled to 2°C to 3°C until use.

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the different concentration of (20-100) µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any drug sample. It can be calculated according to the following formula:

Percentage inhibition= Absorption control – Absorption sample × 100/Absorption control

***Salivary enzymes assay:***

Saliva samples were collected from the rabbit mouth with help of cotton and dissolve in 0.1 M Tris buffer (pH 7.0), proper carefully handling the animal and saliva obtained to help of rabbit mouth guard and saliva secretion flow rate increased to used sialogogue drug dose given to rabbit. It was centrifuged at 13,000 rpm for 20 min. An aliquot of the supernatant was diluted with water. The collected supernatant containing salivary enzyme was assayed by the method reported previously with a slight modification. The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the deferent concentration of (20-100) µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any drug sample. It can be calculated according to the following formula:

Percentage inhibition= Absorption control – Absorption sample × 100/Absorption control

***Fungal α-amylase inhibitory activity:***

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in concentration range 20-100 µg/ml were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm.[26] The control samples were prepared without any drug sample. It can be calculated according to the following formula:

Percentage inhibition= Absorption control – Absorption sample × 100/Absorption control

***Sucrase enzyme inhibition activity:***

Isolated rat intestine was chilled with ice cold 80 mM phosphate buffer. The intestine was then cut open, the mucosa scraped off with a piece of glass rod and homogenized with four parts (v/v) of cold buffer. Nuclei and large cell debris were removed by centrifugation at 2000 to 4000 rpm for 10 mins and supernatant aliquoted into 1.5 ml vials and stored at -20 ºC. Enzyme inhibitory assay was performed according to the method of Honda and Hara. The enzyme solution 10µl and varying concentrations of the test sample (20-100µg) were incubated together for 10 minutes at 37 °C, and the volume was made up to 200 µL with maleate buffer (pH 6.0). The enzyme reaction was started by adding 100 µl sucrose solution (60 mM). After 30 minutes, the reaction was terminated by adding 200 µL of 3, 5-dinitrosalicylic acid reagent and treating the mixture in a boiling water bath for 5 minutes. The absorbance of the solution was read at 540 nm.It can be calculated according to the following formula:

Percentage inhibition= Absorption control – Absorption sample × 100/Absorption control

Where, Absorbance control is the absorbance of the control reaction (containing all reagents except the test sample), and the Absorbance sample is the absorbance of the test sample. An untreated enzyme solution was used as the control. All the experiments were carried out in triplicate.

***Alpha- glucosidase enzyme inhibition activity:***

α-glucosidase type IV enzyme obtained from *B. stearothermophilus*was dissolved in 0.5 M phosphate buffer (pH 6.5) at the rate of 3 U/ml.The α-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from p-nitrophenyl α-D glucopyranoside. The assay mixtures for these experiments contained 0.3 ml of 10mM p-nitrophenyl α-D-glucopyranoside, 1.0 ml of potassium phosphate (0.1M, pH: 6.8), 0.2 ml of enzyme solution and 0.2 ml of inhibitor test sample , all in a final volume of 1.7 ml. Following an incubation time of 30 min at 37º C, the reaction was terminated by the addition of 2.0 ml of 100 mM sodium carbonate. [152] The liberated p-nitrophenol was determined at 400 nm using spectrophotometer.The % inhibition rates were calculated using the formula,

Inhibition (%) = 100(Absorbance Control - Absorbance Test/Absorbance Control)

Suitable reagent blank and inhibitor controls were also carried out and subtracted. Dose dependent variation in the α-glucosidase inhibition was measured using 25 µl to 200 µl of the aqueous extracts. (A110)(A105)

**Method of α-glucosidase rat small intestine:**

Rat small intestine homogenate was prepared according to the method described by Loss w*et al.,* (1964) with slight modifications. The small intestine of male Wistar rats weighing 180g was collected after sacrificing the animal under anesthesia. The Small intestine was removed and washed with saline (30ml of 0.9% NaCl) and epithelial layer (mucosal tissue) was collected by scraping the luminal

surface firmly with a spatula and placed in ice cold 0.9% NaCl. The intestine was minced with a surgical knife and homogenized using in 50 ml of 0.1 M potassium phosphate buffer of pH 6.8. After 30 min, the homogenates were centrifuged for 30 min at 10,000 rpm at 4°C. The supernatant fraction contained rat small intestinal α-glucosidase. The Supernatant was used as crude enzyme source. (A104)Alpha-glucosidase inhibitory activity was assayed according to the method of Honda and Hara. Enzyme solution (10 µL) and three fractions of test sample (10–50 µL) were incubated together for 10 min at 37°C and the volume was made up to 210 µL with maleate buffer, pH 6.0. The enzyme reaction was started by adding 200 µL of 2 mMp-nitrophenyl-α-d-glucopyranoside solution and further incubated at 37°C for 30 min. The reaction was terminated by treating the mixture in a boiling water bath for 5 min. After the addition of 1.0 mL of 0.1 M disodium hydrogen phosphate solution, absorption of the liberated p-nitrophenol was read at 400 nm. Enzyme inhibition assay was measured based on solving the substrate to produce colored products. (7)It can be calculated according to the following formula:

Percentage inhibition= Absorption control – Absorption sample × 100/Absorption control

**Assay of Alpha-glucosidase from baker's yeast:**

The yeast glucosidase was dissolve in 100 mM phosphate buffer, pH 6.8 was used as enzyme source. 100mMP-nitrophenyl-α-d-glucopyranoside was used as substrate. The test sample was weighted and dissolves with water to get a concentration of 20-100 µg/ml. the test sample was mixed with 320 µl of 100mM phosphate buffer (pH 6.8) and 50 µl of 10mM PNPG in the buffer and then it was incubated at 30º C for 5 minutes. After the incubation, 20 µl of the buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 30º C for 5 minutes. Finally, 3.0 ml of 50 mM sodium hydroxide was measured at 410 nm on a spectrophotometer.The enzyme without test sample was used as a control.The IC50 values were determine from plots of percent inhibition Vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.[27]

## RESULTS

Table – 1 shows wheat alpha-amylase activity of neohesperidin. Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 55.17% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 34.13, 65.85 and 82.64 µg/ml. Figure – 1 shows standard curve for acarbose. Table – 2 shows salivary alpha-amylase activity of neohesperidin. Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 48.77% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 32.97, 34.22 and 40.65µg/ml. Figure – 2 shows standard curve for acarbose. Table – 3 shows fungal alpha-amylase activity of neohesperidin. Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 25.25% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 37.00, 80.20 and 58.21µg/ml. Figure – 3 shows standard curve for acarbose. Table – 4 shows rat small intestine from alpha-glucosidaseof neohesperidin. Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 22.98% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 39.13, 59.62 and 73.03µg/ml. Figure – 4 shows standard curve for acarbose. Table – 5 shows alpha-glucosidase inhibitory activity of neohesperidin obtained from *B. stearothermophil.* Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 8.51% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 466.21, 418.29 and 554.59µg/ml. Figure – 5 shows standard curve for acarbose. Table – 6 shows alpha-glucosidase inhibitory activity of neohesperidin obtained from baker's yeast. Neohesperidin inhibited alpha-amylase dose dependently. The percentage inhibition of neohespiridin was 51.55% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 63.23, 79.09 and 57.02µg/ml. Figure – 6 shows standard curve for acarbose. Table – 7 shows inhibitory activity of neohesperidine on sucrase from rat intestine. Neohesperidin inhibited surase dose dependently. The percentage inhibition of neohespiridin was 61.11% at highest concentration (100 µg/ml). $IC_{50}$ value of acarbose, diabecon and neohesperidin was 32.22, 49.78 and 87.69 µg/ml. Figure – 7 shows standard curve for acarbose.

**Table no. – 1** Wheat alpha-amylase activity of neohesperidin

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$(µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| 1. | 20 | 47.58±3.47 | 34.02±2.19 | 30.11±3.19 | 34.13 | 65.85 | 82.64 |
| 2. | 40 | 48.36±0.83 | 41.84±2.65 | 38.85±0.82 | | | |
| 3. | 60 | 60.45±2.79 | 45.51±0.39 | 42.3±1.61 | | | |
| 4. | 80 | 67.12±4.25 | 52.87±2.93 | 49.42±2.82 | | | |
| 5. | 100 | 77.93±2.1 | 65.28±4.47 | 55.17±4.15 | | | |

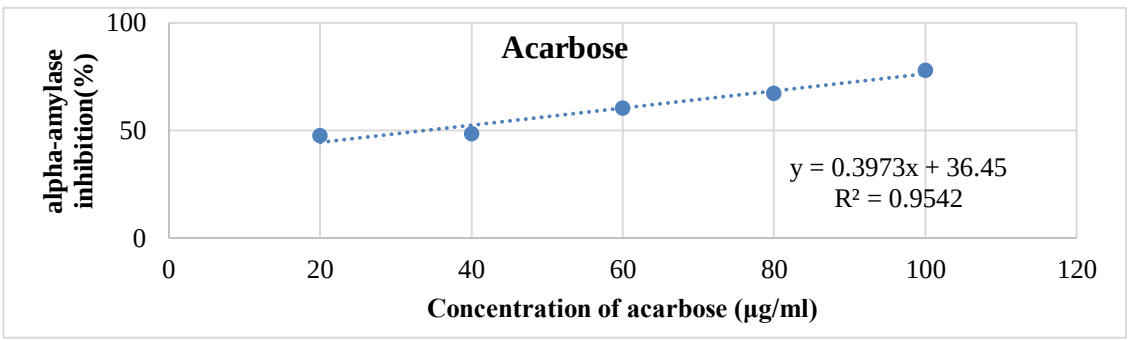


**Fig. no. 1:** Wheat alpha-amylase (Acarbose).

**Table no. – 2** Salivary alpha-amylase activity of neohesperidin

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| 1. | 20 | 52.45±8.29 | 40.34±10.67 | 37.71±6.14 | 32.97 | 34.22 | 40.65 |
| 2. | 40 | 55.61±4.5 | 49.99±4.02 | 49.12±3.16 | | | |
| 3. | 60 | 70.87±4.7 | 52.63±5.26 | 65.28±2.32 | | | |
| 4. | 80 | 69.26±2.74 | 62.27±7.79 | 70.17±2.31 | | | |
| 5. | 100 | 73.86±0.88 | 60.52±3.98 | 48.77±6.63 | | | |

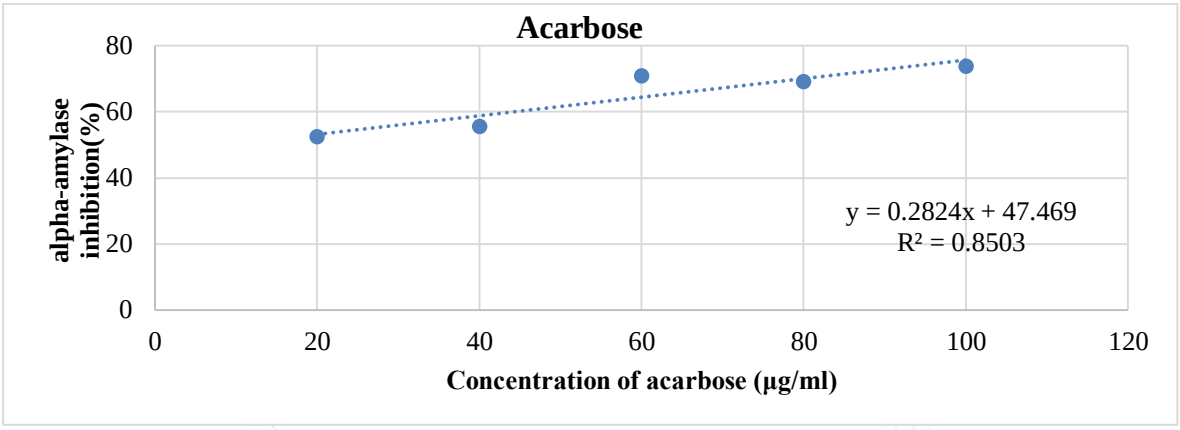


**Fig. no. - 2:** Salivary alpha-amylase (Acarbose)

**Table no. – 3** Fungal alpha-amylase activity of neohesperidin

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| | 20 | 47.59±4.93 | 44.97±6.2 | 25.11±4.55 | 25.25 | 37.00 | 80.20 |
| | 40 | 56.72±1.68 | 51.36±4.55 | 36.3±1.72 | | | |
| | 60 | 59.81±3.22 | 57.07±2.96 | 41.78±1.72 | | | |
| | 80 | 68.94±6.2 | 60.95±3.81 | 49.08±2.38 | | | |
| | 100 | 74.65±4.45 | 69.63±3.07 | 58.21±3.37 | | | |

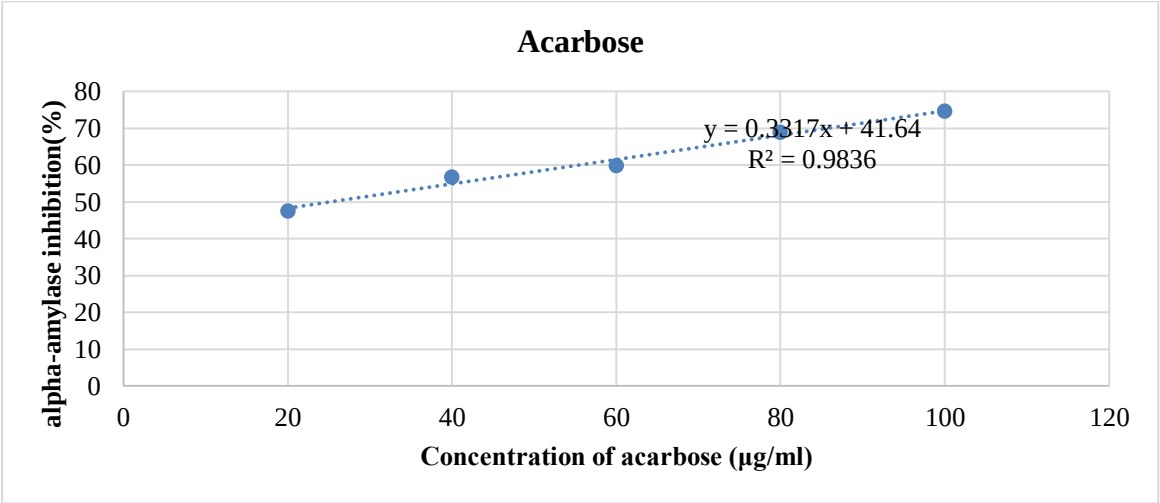


**Fig. no. - 3:** alpha-amylase (Acarbose)

**Table no. – 4** Rat small intestines from alpha-glucosidase

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| | 20 | 53.07±2.99 | 43.94±3.67 | 28.65±4.41 | 22.98 | 39.13 | 59.62 |
| | 40 | 60.08±4.59 | 49.04±3.67 | 38.21±1.32 | | | |
| | 60 | 63.47±6.62 | 55.83±5.42 | 46.28±1.88 | | | |
| | 80 | 74.09±3.7 | 69.42±7.15 | 65.2±1.12 | | | |
| | 100 | 80.67±2.95 | 77.49±1.7 | 73.03±1.88 | | | |

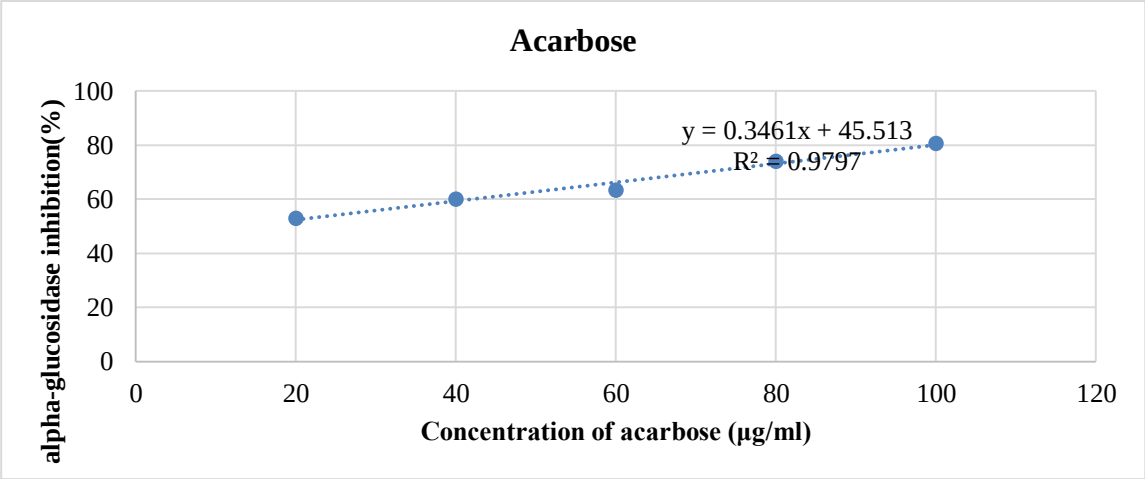


**Fig. no. - 4:** Rat small intestine from alpha-glucosidase (Acarbose)

**Table no. – 5** Alpha-glucosidase from *B. stearothermophil*

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| | 20 | 6.24±0.31 | 2.99±0.51 | 1.3±0.21 | 466.21 | 418.29 | 554.59 |
| | 40 | 7±0.38 | 5.5±0.22 | 3.25±0.21 | | | |
| | 60 | 9.93±0.78 | 7.69±1.4 | 4.9±0.66 | | | |
| | 80 | 12.33±0.63 | 10.03±0.85 | 7.09±0.95 | | | |
| | 100 | 13.5±1.26 | 12.59±0.96 | 8.51±1.14 | | | |

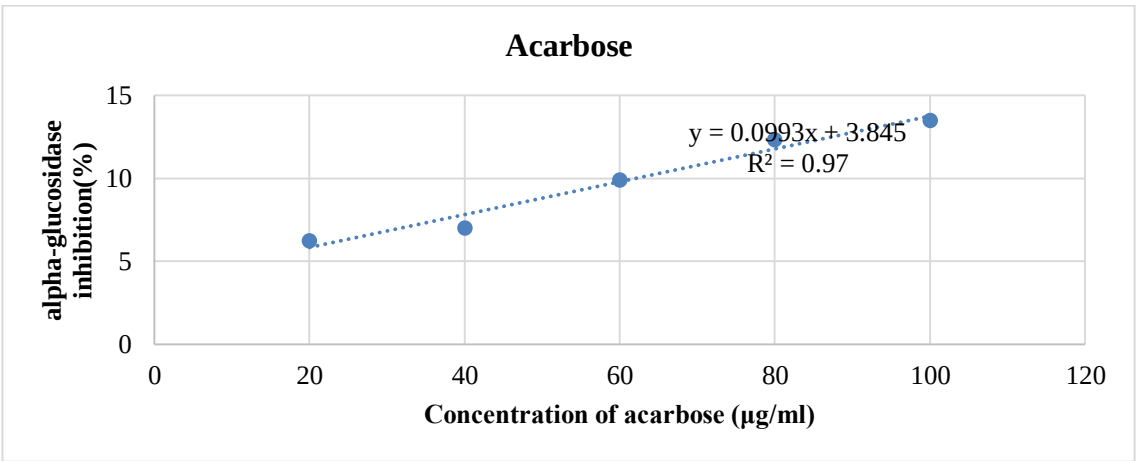


**Fig. no. - 5:** Alpha-glucosidase from B.stearothermophil (Acarbose)

**Table no. – 6** Alpha-glucosidase from baker's yeast

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| 1 | 20 | 27.12±4.39 | 10.78±2.46 | 6.86±3.53 | 57.02 | 63.23 | 79.09 |
| 2 | 40 | 39.54±7.21 | 19.6±0.56 | 14.7±4.42 | | | |
| 3 | 60 | 52.61±7.07 | 33.33±1.13 | 26.14±6.23 | | | |
| 4 | 80 | 63.72±5.96 | 47.05±3.14 | 36.27±5.4 | | | |
| 5 | 100 | 76.14±2.9 | 66.42±4.3 | 51.55±5.84 | | | |

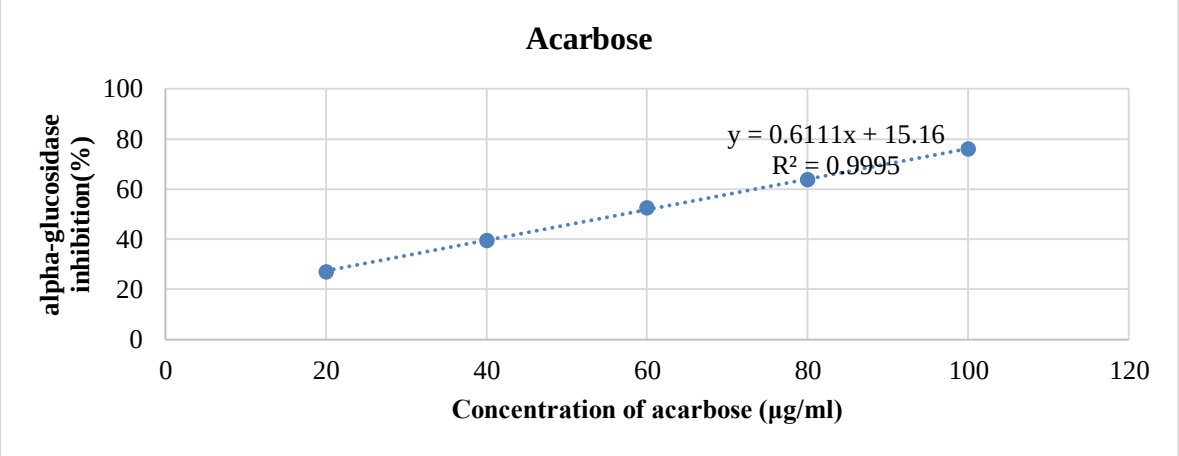


**Fig. no. - 6:** Alpha-glucosidase from baker's yeast (Acarbose)

**Table no. – 7** Sucrase from rat small intestine

| S. No. | Concentration (µg/ml) | Percentage inhibition | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|---|---|
| | | Acarbose | Diabecon | Neohesperidin | Acarbose | Diabecon | Neohesperidin |
| 1. | 20 | 42.3±8.72 | 26.01±6.68 | 11.96±1.54 | 32.22 | 49.78 | 87.69 |
| 2. | 40 | 54.42±5.74 | 45.73±7.99 | 25.63±4.62 | | | |
| 3. | 60 | 66.65±4.43 | 61.1±6.67 | 29.05±3.33 | | | |
| 4. | 80 | 78.2±2.67 | 72.64±7.1 | 42.64±3.68 | | | |
| 5. | 100 | 86.74±1.13 | 78.2±4.5 | 61.11±1.54 | | | |

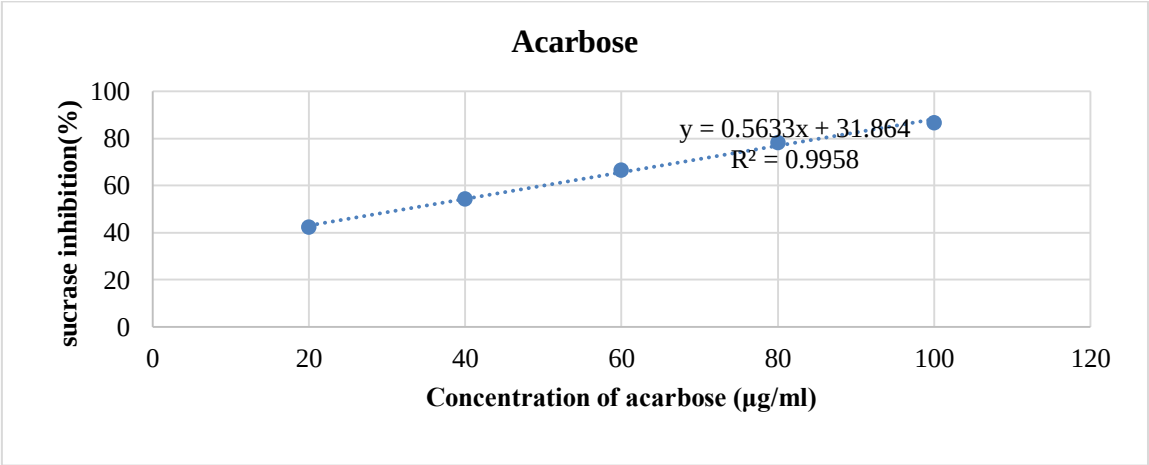


**Fig. no. - 7:** Sucrase from rat small intestine (Acarbose)

## DISCUSSION:

According to the results obtained at different concentrations, we observed that the anti-diabetic inhibitory activities of the enzyme which hydrolyze carbohydrates depends on the concentration of Neohesperidin pure compound. Result observed by neohesperidin on different enzymes are the alpha-amylase (wheat alpha-amylase, salivary amylase and fungal amylase.), alpha-glucosidase (rat small intestine glucosidase, baker's yeast, B.stearothermophil) and Sucrase rat intestinal enzyme that is showed better inhibitory activity as compare to alpha-glucosidase and Sucrase.

Inhibition of enzymes involved in the hydrolysis of carbohydrates such as alpha amylase & alpha glucosidase has been exploited as a therapeutic approach for controlling postprandial hyperglycemia. Alpha glucosidase (intestinal alpha glucosidase) catalyzes the breakdown of disaccharides to liberate glucose which is later absorbed into the blood circulation. Inhibition of these enzymes would slow down the breakdown of starch in the gastro-intestinal tract, thus reducing postprandial hyperglycemia.

In recent diabetic treatments, α-amylase and α-glucosidase inhibitors are most potented because they increase post-prandial hyperglycemic conditions. The antioxidant ability of phenolic compounds in fruits and vegetables could be attributed to their properties such as reducing agents, hydrogen donors, singlet hydrogen quenchers and/or metal ion- cheaters. Therefore, natural antioxidants can also inhibit the key enzymes α-amylase, α-glucosidase and control the post-prandial hyperglycemic conditions which are a potential approach to cure the type 2 diabetes mellitus.

Flavonoids are a prominent group of secondary metabolites in citrus fruits that may possess biological activity and have beneficial effects on human health as antimicrobial, anti-inflammatory, anti-diabetic, anti-cholesterolemic, antioxidant and anti-cancer agents. Flavonoids are reported to possess strong free radical scavenging activities based on their ability to act as hydrogen or electron donors and chelate transition metals.

Among the ubiquitous flavonoids found in the plant kingdom, NHP is one of the several flavanones glycosides unique to Citrus and specific citrus cultivars. Studies have shown that supplemental natural phenolic from different fruit sources represent a potential strategy as adjunct therapy. Recently, extensive studies of citrus flavanones have been reported to have potential hypoglycaemic effect.

Neohesperidin were found to be the most abundant flavonoids in grapefruit and Seville orange (C. aurantium), and their concentration can be used to differentiate orange juices from grapefruit and sour orange sources. Huyou fruit is most likely a hybrid between sour orange and pummelo, both of which have a bitter taste.

In the present study simultaneous purified neohesperidin purchased from sigma-aldrich neohesperidin are reported in previous review literature studies showed anti-oxidant, anti-diabetic and anti-lipidemic activities. *In-vitro* carbohydrate metabolizing enzyme based inhibitory method used. In above experimental study results have been showed that enzyme inhibitory activity such as neohesperidin were shown to have a significantly higher inhibitory activity on alpha-amylase with compare to alpha-glucosidase & Sucrase.

Recently the Neohesperidin showed a significant inhibiting both alpha-amylase and alpha-glucosidase. The alpha-amylase different sources are wheat alpha-amylase $IC_{50}$-82.64, salivary alpha-amylase $IC_{50}$-40.65and fungal alpha-amylase $IC_{50}$-80.20. This result concluded that

Salivary alpha-amylase > fungal alpha-amylase > wheat alpha-amylase.

Alpha-glucosidase obtained from different source is rat small intestine alpha-glucosidase $IC_{50}$-59.62, B. stearothermophil $IC_{50}$-554.59, baker's yeast $IC_{50}$-79.09. Result calculated that

Rat small intestine alpha-glucosidase > B.stearothermophil > baker's yeast.

Sucrase is significant result shown $IC_{50}$-87.69 according to the results higher inhibitory activity shown Neohesperidin in alpha-amylase and less weak inhibitory activity on alpha-glucosidase and Sucrase. The results obtained at different concentrations, we observed that the anti-diabetic inhibitory activities of the enzymes which hydrolyze carbohydrate depend on the concentration of flavonoids present in the Neohesperidin pure compound.

Diabetes is characterized by high concentrations of blood sugar which can cause serious complications in the kidneys, eyes and cardiovascular system. The treatment of diabetes there-fore mainly focuses on reducing fluctuations in blood sugar and subsequent complications. The α-amylase and α-glucosidase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents. Acarbose is a commercially available enzyme inhibitor for type II diabetes. However, it is reported to cause various side effects such as abdominal distention, flatulence and possibly diarrhea. Searching for safe and effective inhibitors from natural sources are of emerging interest. The inhibitory effects of the neohesperidin, acarbose as a standard established anti-diabetic drug and marketed herbal formulation are Diabecon (Himalaya tablet formulation) are used to inhibitory activity comparison neohesperidin on α-amylase, α-glucosidase and Sucrase. Than result obtained neohesperidin acarbose and Diabecon herbal anti-diabetic drug are nearby activity showed.

## CONCLUSION:

In present study pure Neohesperidin Phytoconstituent purchased from Sigma-Aldrich, Japan. Early reported that it's having a potent anti-oxidant, anti-diabetic and anti-lipidemic. In above *In-vitro* experiment carbohydrate metabolizing enzyme based inhibitory methods used. It was concluded that if so better activity in alpha-amylase as compare to alpha-glucosidase and Sucrase.

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