EXPERIMENT 11

AIM

To detect the presence of sugar in urine.

THEORY

Urea is the major excretory product present in urine of mammals. Normal urine does not contain glucose. The presence of glucose in urine is an abnormality and called glucosuria. There are three main tests, i.e. Benedict's test, Fehling's test and Seliwanoff's test, which are used for detection of sugars like glucose, fructose, etc. in urine. These tests are sensitive between 50-80 mg glucose per 100 ml of urine. Less than this amount in the urine goes undetected by these tests. When sugar level increases in blood due to untreated diabetes mellitus, glycosuria is caused.

MATERIAL REQUIRED

Test tubes, beakers, test tube stand, test tube holder, spirit lamp, pipette, urine sample, Benedict's reagent, Fehling's solution A, Fehling's solution B and Seliwanoff's reagent.

PROCEDURE AND OBSERVATIONS

1. **Benedict's Test**
   - **(i)** Take 0.5 ml of freshly passed urine sample in a test tube and add 5 ml of Benedict's reagent into it.
   - **(ii)** Boil the solution for 2 minutes holding the test tube firmly with a test tube holder in inclined position and keep shaking the tube continuously as the contents of the test tube have a tendency to spurt out.
   - **(iii)** Note down the colours of the precipitate.
2. **Fehling's Test**
   - **(i)** Take equal volumes (2 ml) of Fehling's solutions A and B in a test tube and mix them well.
   - **(ii)** Add the solution prepared (i) drop wise to 1 ml of urine sample taken in another test tube.
   - **(iii)** Heat the solution for 2 minutes with continuous shaking.
   - **(iv)** Observe the colour of the precipitate.
3. **Seminoff's Test**
   - **(i)** Take 3 ml Seliwanoff's reagent in a test tube and add 1 ml of urine sample to it.
   - **(ii)** Boil the tube for 2 minutes in water bath.
   - **(iii)** Appearance of red or orange colour indicates the presence of fructose.
   - **(iv)** If no colour appears in 2 minutes, continue boiling for 5 minutes.
   - **(v)** If faint orange or no colour appears even after (iv) step, the presence of glucose is considered confirmed.

Note: Seliwanoff's test is to be performed when urine sample gives positive test for Benedict's or Fehling's test.

OBSERVATION.

1. **Benedict's test:** Green, yellow light green and brick-red coloured precipitate appears, which indicates the presence of reducing substances in urine. The various coloured precipitates depend on the concentration of reducing sugar in the urine sample as follows:

S.No.	Colour of Precipitate	% Of Reducing Sugar Present
1.	Light green	0.1-0.5 (traces of glucose)
2.	Green	0.5 - 1 (moderate amount of glucose)
3.	Yellow	1.0-2.0 (moderate amount of glucose)
4.	Brick-red	above 2 (large amount of glucose)

2. **Fehling's test:** A yellow or orange or brick-red coloured precipitate is formed, which indicates the presence of reducing sugars in urine.
3. **Seliwanoff's test:** Presence of red or orange colour gives positive test for fructose, while appearance of faint orange or no colour gives the positive test for glucose.

RESULT

The given sample of urine contains reducing sugar such as glucose, fructose, etc.

PRECAUTIONS

1. The chemicals should be poured or used in a proper amount to avoid the false positive results.
2. Standard reagents and chemicals must be used.
3. Do not mix the droppers used for pouring solutions or reagents to avoid contamination.
4. Proper care must be taken while heating the test tube.
5. One must use gloves, while working with the urine sample to keep themselves protected from infections.

VIVA VOCE

Q1. What is meant by glucosuria?

Ans. Excretion of glucose with the urine is called glucosuria.

Q2. What is the cause of glucosuria?

Ans. When excess of sugar is present (in case of uncontrolled diabetes) glucose is excreted through urine.

Q3. What do you mean by renal diabetes?

Ans. It is a condition in which the kidney threshold for glucose is below normal, but the blood sugar is normal.

Q4. Give a difference between diabetes mellitus and diabetes insipidus

Ans. **Diabetes mellitus** is caused when sugar level increases in blood and appears in urine due to the deficiency of insulin hormone.

Diabetes insipidus is caused when water level increases in urine and most of the water is excreted out in urine due to the deficiency of Anti-diuretic Hormone (ADH).

Q5. Name the disorder in which glucose level is high in urine

Ans. Glycosuria is the disorder in which glucose level is high in urine.

Q6. What is the significance of appearance of different colours, while performing Benedict's test?

Ans. The appearance of different colours, while performing Benedict's test indicates or gives the idea about the quantity of sugar present in the solution, e.g. greenish precipitate indicates the presence of glucose in traces, while red precipitate indicates the presence of higher concentration of glucose in urine.

Q7. What is the significance of performing Seliwanoff's test?

Ans. Benedict's test and Fehling's test may give false positive test due to the presence of drugs and contaminants in the urine. Also these tests are positive for other reducing sugars (e.g. lactose. maltose.) Therefore, Seminoff's test is performed for detecting the presence of glucose or fructose in the urine. It is a confirmatory test.

Q8. What is the end product of Benedict's and Fehling's tests?

Ans. The coloured insoluble precipitate is the end product of Benedict's and Fehling's tests.

Q9. What is the basic principle of Benedict's and Fehling's test?

Ans. The basic principle of Benedict's and Fehling's solution is that glucose or any other reducing agent reduces the blue cupric sulphate of Benedict's and Fehling's reagents to a coloured insoluble precipitate.

Q10. What is the significance of the urine test?

Ans. It detects the pathological conditions of a person. So that proper treatment can be given by a doctor.