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***Research Article** (Open Access)*

COMPARATIVE STUDY OF ANTI-INFLAMMATORY ACTIVITY OF *VIGNAMUNGO* IN PCOS BLOOD SAMPLE AND NON-PCOS BLOOD SAMPLE

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ABSTRACT
Polycystic ovary syndrome (PCOS) is a hormonal disorder common among Women of reproductive age. Urad dal (*Vigna mungo)* helps in lowering inflammation due to its antiinflammatory properties. Benefits with respect to the female reproductive system. It is Used in the treatment of painful menstruation, amenorrhea, and PCOS. The blood samples, one from a person with PCOS and one from a person without PCOS are taken. After processing, the optical density of the samples obtained using a Spectrophotometer was noted and the percentage of hemolysis inhibition was calculated and noted the percentage of hemolysis inhibition caused due to the presence of the *Vigna mungo* seed extract and the *Vigna mungo* seed coat extract was noted where the inhibition was caused due to the anti-inflammatory effects of *Vigna mungo. The* results were analyzed and presented in the form of graphical representation.The percentage of inhibition in the samples was more than 50%, which showed that the
anti-inflammatory effect of the extracts in the samples was higher. The inhibition was seen to be higher in the presence of *Vigna mungo* seed coat. Extract than the *Vigna mungo* seed extract comparatively. From the experiment, it is concluded that the hemolysis inhibition is more in the PCOS blood sample than in the Control blood sample in the presence of the seed extract. This is due to the anti-inflammatory effect of the *Vigna mungo* which prevents the Destruction of RBCs. From the experiment, it is concluded that the hemolysis inhibition is Comparatively less in the PCOS blood sample than in the Control blood sample in the Presence of the *Vigna mungo* seed coat extract.
Comparing the values obtained from both the *Vigna mungo* seed extract and the *Vigna mungo* seed coat it is clear that the *Vigna mungo* seed coat shows a higher Hemolysis inhibition than the seed**.**

Keywords: *PCOS, Vigna mungo, Hemolysis inhibition, Blood Samples, Anti-inflammation*

INTRODUCTION
In India, one out of five women is suffering from PCOS (Polycystic Ovary Syndrome). Generally, PCOS is caused due to an imbalance of hormones and increased secretion of androgens, which leads to the formation of cysts in the ovaries. According to records, 50% of women who suffer from PCOS go undiagnosed and do not have proper treatment. The main reason for this is, assuming irregular menstruation as the major symptom of PCOS, but it is not known that Women with PCOS can also have regular menstrual cycles.
A diet adapted to PCOS, and some lifestyle changes can improve and decrease some of the symptoms associated with PCOS. The foods that have improved the symptoms are
1. High fibre vegetables like Legumes, Wholegrains, Nuts, Avocados, Pears, Berries, Coconut, and Peas.
2. Lean protein, like Lentils, fish, Spinach, Kale, Parsley, Chia seeds, Walnuts, Oats, Black beans, green peas., etc.
3. Anti-inflammatory foods and spices like Berries, Fatty fish, Cruciferous vegetables, green tea, Tomatoes, Olive oil and coconut oil, Turmeric, and Pepper

1.1 *PCOS:*
Polycystic ovary syndrome (PCOS) is the most common endocrinopathy, affecting 5–13% of women of reproductive age. In adolescents, depending on the criteria used, PCOS is diagnosed in 3.4%, 8%, and 11% (Teede.H.; *et al.*, 2010). It is considered the most prevalent endocrine disorder among menstruating women

and is characterized by features that suggest varying combinations of functional reproductive shortage (such as ovulatory dysfunction) and androgen excess (such as acne and hirsutism). At present, PCOS is commonly diagnosed based on symptoms such as acne, hirsutism, and irregular menstrual cycles, followed by targeted screening. Identification of PCOS, at an early stage, minimizes the overall health risk and fertility-related issues that the person might endure in the future.

Pathophysiology: The disordered physiological processes associated with PCOS are Abnormal ovarian morphology are Approximately six to eight times more pre-antral and small antral follicles are present in the polycystic ovary compared with the normal ovary. (Webber LJ 2003) The arrest in development at a size of 2– 9 mm, has a slow rate of atresia, and is sensitive to exogenous follicle-stimulating hormone (FSH).

Excessive ovarian androgen production: Almost every enzymatic action within the polycystic ovary that encourages androgen production is accelerated. Both insulin and LH, alone and in combination, exacerbate androgen production.

Hyperinsulinemia: Its due to insulin resistance occurs in approximately 80% of women with PCOS and central obesity, but also in approximately 30–40% of lean women with PCOS.8 This is thought to be due to a post-receptor defect affecting glucose transport and is unique to women with PCOS. (Dunaif,1997)

Excessive serum concentrations of LH: Excessive serum concentrations of LH are detected on single-spot blood samples in approximately 40–50% of women with PCOS. Although FSH serum concentrations are often within the low normal range, an intrinsic inhibition of FSH action may be present. Prolactin concentrations may be slightly elevated.

1.2 DIAGNOSIS:

The three recognized sets of criteria for PCOS diagnosis include the

- NIH (National Institutes of Health) criteria
- Rotterdam criteria, and
- Androgen Excess and Polycystic Ovary Syndrome Society criteria.

The National Institutes of Health criteria require evidence of hyperandrogenism and menstrual irregularity, without knowledge of ovarian findings. The European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine criteria, often called Rotterdam, includes ultrasound findings of a polycystic ovary and allows for various phenotypes based on a combination of any 2 of the 3 findings of hyperandrogenism, menstrual irregularity, and polycystic ovaries on ultrasound. Finally, criteria set forth by the Androgen Excess and PCOS Society stress hyperandrogenism, with the associated findings of either menstrual irregularity or polycystic ovaries on ultrasound (Enrico Carmina *et al.*, 2010).

The Rotterdam criteria is the most widely used for diagnosis. It defines PCOS as the presence of at least two ovulatory dysfunctions, hyperandrogenism, and polycystic ovarian morphology. Before diagnosing PCOS, other potential diagnoses including thyroid disorders, hyperprolactinemia, congenital adrenal hyperplasia, and Cushing's syndrome should be ruled out. The prevalence of PCOS depends upon the diagnostic criteria used. Rotterdam is most inclusive, followed by the Androgen Excess and Polycystic Ovary Syndrome Society criteria. The NIH criteria are the most strict and therefore the prevalence of PCOS is the lowest.

1.3 SYMPTOMS AND CAUSES:

Polycystic ovarian syndrome (PCOS) occurs when the female reproductive organ produces unusually high levels of hormones called androgens, which leads to hormonal imbalance. PCOS has been known to be associated with irregular periods, infertility, increased pregnancy complications, as well as nonreproductive health problems arising from its association with metabolic syndrome (Shunping Wang, *et al.*, 2013)

Further, the following causes are also considered to be responsible for disturbing the hormonal balance:

1. Lack of ovulation
2. Insulin resistance
3. Inflammation
4. Genes
5. Obesity
6. Hypothyroidism

Apart from the above said reasons, the following aspects of a person's lifestyle such as

1. Laziness (lack of exercise, sedentary lifestyle).
2. Fast food, processed food - with lots & lots of sugar and salt in them, also stale and fried food.
3. Stress (mental stress, environmental stress, work- stress).
4. Insomnia (improper/disturbed night sleep, not being able to sleep until late, lack of sound- sleep, sleeping during the daytime).
5. Less water intake, also gives rise to hormonal imbalance.

A person showing the following symptoms can be at risk of being diagnosed with PCOS

- Missed periods, irregular periods, or very light periods
- Excess body hair, including the chest, stomach, and back (hirsutism)
- Weight gain, especially around the belly (abdomen)
- Acne or oily skin
- Male-pattern baldness or thinning hair
- Infertility
- Small pieces of excess skin on the neck or armpits (skin tags)
- Dark or thick skin patches on the back of the neck, in the armpits, and under the breasts

And on further inspection, this can be clinically confirmed by the presence of ovaries that are large and /or with cysts.

1.4 COMPLICATIONS

PCOS is a major cause of infertility in a woman and reports show that about 20% of the female population of India is diagnosed with PCOS. Even those who get pregnant despite having PCOS face a high risk of miscarriage, premature birth, Gestational diabetes, or pregnancy-induced high blood pressure. Further due to irregular periods if one doesn't ovulate every month, the inner lining of the ovary can build up leading to Endometrial cancer.

PCOS women have multiple risk factors for diabetes including obesity, a family history of type 2 diabetes, and abnormalities in insulin action (both insulin resistance and b-cell dysfunction). There is no clear evidence that women with PCOS are at an increased (3±7 times) risk of developing type 2 diabetes (Dunaif *et al.*, 1987; Wild *et al.*, 2000). Of the patients with confirmed PCOS, almost one-third had uterine anomalies. Further, Obesity is associated with PCOS and can worsen complications of the disorder. Metabolic syndrome (a cluster of conditions including high blood pressure, high blood sugar, and abnormal cholesterol or triglyceride levels that significantly increase your risk of cardiovascular disease), Sleep apnea, Depression, anxiety, and eating disorders are some of the common complications experienced by a person diagnosed by PCOS.

1.5 DIETARY PREFERENCE FOR PCOS

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder. Most women with PCOS, regardless of weight, have a form of insulin resistance that is intrinsic to the syndrome. Obese women with PCOS have an added burden of insulin resistance related to their adiposity. Thus, adequate nutritional status is a critical determinant of the onset and maintenance of normal reproductive function.

Weight loss should always be recommended as a first-line approach in the treatment of obese and overweight PCOS women, since it significantly improves hormonal and metabolic abnormalities, and may favor spontaneous ovulation improving fertility in most patients. In addition, weight loss associated with moderate physical activity is desirable because physical exercise improves the reduction of insulin resistance (Palomba *et al.*, 2014)

PCOS studies show that a high-protein/low-carb diet:

- Help boost metabolism: The thermic effect of protein is between 15 and 30%, which is far greater than that of carbohydrates (5 to 10%) or fats (0 to 3%).
- Control appetite: Protein stimulates the production of cholecystokinin, glucagon, and hormones that are involved in helping to reduce appetite.

- Improve blood sugar control: Due to the slower digestion of protein, its impact on blood sugar is relatively low.

Moderate insulin response: Protein stimulates the release of glucagon, a hormone that raises blood glucose levels and counteracts the action of insulin.

- The right amount of protein can help balance the levels of glucagon and insulin in the blood.

1.6 BLACK GRAM - *Vigna mungo*

Legume seeds are the most important source of protein and calories for large segments of the population mainly in India. Black gram or urad dal holds a higher protein value than most legumes (Yamez-Farias, 1997). It contains on average 10.9% moisture, 24% protein, 1.4% fat, 0.9% fiber, and 59.6% carbohydrate as the main component. It has been reported that the starch content of black gram is 47.9% with an amylose content of 43.8% (Srinivasa Rao, 1976).

It is also an excellent source of dietary fiber, isoflavones, vitamin B complex, iron, copper, calcium, magnesium, zinc, potassium, and phosphorus which offers a myriad of healing health benefits. Black gram is an important legume crop throughout a large part of the tropics.

1.7 NUTRITIONAL VALUE

According to the United States Department of Agriculture, the nutritional value of Vigna mungo is

VIGNA MUNGO - SEED

Energy	341 Kcal
Carbohydrates	58.99kg
Protein	25.21g
Total fat	1.64g
Dietary Fiber	18.3g
Folates	216mg
Niacin	1.447mg
Pantothenic acid	0.906mg
Pyridoxine	0.281mg
Riboflavin	0.254mg
Thiamin	0.273mg
Vitamin-A	23IU 1%
Sodium	38mg
Potassium	983mg
Calcium	138mg
Copper	0.981mg
Iron	7.57mg
Magnesium	267mg
Phosphorous	379mg
Zinc	3.35mg

VIGNA MUNGO – SEED COAT

Reducing sugar	97.3 ± 8.2
Total carbohydrate	27.5 ± 2.7
Total protein	10.075 ± 0.9
Moisture content	11.034 ± 0.5
Total fat	0.46 ± 0.1
Crude fiber	48.67 ± 2.0
Ash content	4.87 ± 0.3
Calcium	1062.58 ± 17.48
Sodium	523.47 ± 15.11
Magnesium	440.41 ± 13.80
Potassium	304.02 ± 3.58
Ferrous	10.47 ± 1.75
Manganese	6.38 ± 0.87
Copper	1.57 ± 0.05
Zinc	1.15 ± 0.04

(Pushpam Marie Arockianathan et al., 2019)

1.8 HEALTH BENEFITS OF URAD DAL *(VIGNA MUNGO)*

Health benefits of Urad dal (Vigna mungo) with respect to PCOS:

Reproductive health: Consuming urad dal dishes during menstruation reduces abdominal and muscle cramps or dysmenorrhea. It also addresses the problem of the scanty period and regularizes the menstrual cycle**.**

Lowers blood sugar: Urad Dal or black gram is low on the glycemic index at 43. When soaked the glycemic index for every 30 grams stands at 7.6.

Reduces high blood pressure: It is high in potassium food and prevents the constriction of blood vessels and controls high blood pressure. Its rich iron content helps in the increase of red blood cells, thus facilitating the supply of oxygen throughout the body.

Other Health Benefits of Urad dal

Improves Gut health: Eases bowel movement colic and hemorrhoids and improves liver functions.

Strengthening Nervous System: Urad dal provides instant energy thus aiding the nervous system in carrying out its activities. Doctors and nutritionists recommend the consumption of black grams for patients suffering from facial and partial paralysis, nervous debility, etc.

Builds Bone Health: Black gram is loaded with a wide range of minerals including potassium, magnesium, iron, phosphorous, and calcium which play a crucial role in maintaining the density of bones. It is generally recommended for those low on calcium, iron, and other common nutritional deficiencies and for women in a menopausal state for providing extra strength to the bones.

Decreases the risk of cardiovascular diseases: Studies reveal that regular intake of black grams protects cardiovascular health. It lowers the levels of bad cholesterol (LDL) and prevents atherosclerosis.

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MATERIALS AND METHODS

3.1 PREPARATION OF STANDARD

PROCEDURE
Using a lab balance 10mg of metital was measured. The 10mg of metital was placed in a 100ml beaker. 10ml of distilled water was added to the standard. Then using a glass rod the contents were mixed thoroughly and allowed to stand for some time

3.2 PREPARATION OF CONTROL

PROCEDURE
Using a lab balance 10mg of sodium chloride was measured. The 10mg of sodium chloride was placed in a 100ml beaker. 10ml of distilled water was added to the control. Using a glass rod the contents were mixed thoroughly and allowed to stand for some time.

Plate 1: Sodium chloride used for the preparation of control

3.3 PREPARATION OF BUFFER PROCEDURE

SOLUTION A
0.12g of Disodium hydrogen phosphate was measured in lab balance. The 0.12g of Disodium hydrogen phosphate was placed in a 100ml beaker. 100ml of distilled water was added to it. Using a glass rod the contents were mixed thoroughly and allowed to stand for some time.
SOLUTION B
0.16g of Sodium dihydrogen phosphate was measured in lab balance. 0.16g of Sodium dihydrogen phosphate was placed in a 100ml beaker. 100ml of distilled water was added to it. Then using a glass rod the contents were mixed thoroughly.

3.4 PREPARATION OF ISOTONIC BUFFER

PROCEDURE

In a reagent bottle 100ml of disodium hydrogen phosphate was added. Then 100ml of sodium dihydrogen phosphate was added. The two solutions are mixed thoroughly using a glass rod. 0.89g of sodium chloride was measured using the lab balance. The measured sodium chloride was added to the reagent bottle and mixed thoroughly using a glass rod.

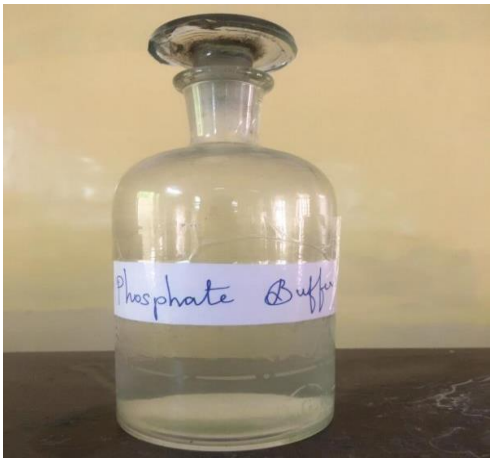

Plate 2: isotonic Buffer

3.5 PREPARATION OF EXTRACT PROCEDURE

Vigna mungo SEED

The seed of black gram without the seed coat was taken to prepare the extract.

The seed was washed thoroughly and then it was allowed to sun dry. The dried seed was made into a fine powder using a grinder and it was sifted to make sure there are no clumps. 10g of this powder was measured using a balance and it was soaked in Ethanol for some time. 500µl of the extract solution was taken using a micropipette and to that 5ml of methanol was added. The solution was allowed to stand for some time.

Vigna mungo SEED COAT

The seed coat of black gram without the seed was taken to prepare the extract.

The seed coat was washed thoroughly and then it was allowed to sundry. The dried seed coat was made into a fine powder using a grinder and it was sifted to make sure there are no clumps. 10g of this powder was measured using a balance and it was soaked in Ethanol for some time. 500µl of the extract solution was taken using a micropipette and to that 5ml of methanol was added. The solution was allowed to stand for some time.

Plate 3: Sun-dried and powdered seed used for the preparation of the extract

Plate 4: Sun-dried and powdered seed coat used for the preparation of the extract

3.6 RED BLOOD CELL SUSPENSION PREPARATION

PROCEDURE

1ml of blood is taken from a person with PCOS and a person without PCOS. The collected blood samples are stored in EDTA tubes till used. 1ml of each blood sample was taken out from the EDTA tubes using a syringe and it was transferred to two separate centrifuge tubes. The blood samples are allowed to centrifuge for 5 minutes at 3000rpm. The plasma was discarded and then 10ml of phosphate buffer was added to both the centrifuge tubes. The samples are centrifuged again for 5 minutes at 3000rpm and the supernatant was discarded. The same procedure was repeated once again and the supernatant was discarded.

To the precipitates, 10ml of phosphate buffer was added and then it was suspended.

Plate 5: Red blood cell suspension obtained after centrifugation

3.7 PROCEDURE FOR ASSAY

PROCEDURE

20µl of standard and 20µl of sodium chloride - saline were added to test tubes marked as S and C. The extract was pipetted out into a series of test tubes labeled as U1, U2, U3, U4, U5, and U6 with the concentration of 20, 40, 60, 80, 100, 120µg respectively. To all the test tubes from U1-U6 and S & C, 2ml of phosphate buffer solution was added. To all the test tubes 200µl of blood was added and it was incubated in a water bath at 56°C for 30 minutes and it was cooled in tap water. After the test tubes are cooled the samples are centrifuged for

5minutes at 2500rpm. The absorbance of the supernatant at 560 nm was noted. The procedure was repeated for all 4 samples. The values are noted and the percentage of hemolysis inhibition was calculated.
Two different blood samples were collected and used for analysis. They are:

- Blood sample from a person with PCOS
- Blood sample from a person without PCOS

These blood samples were treated with 2 different extracts each:
1. PCOS Blood and Urad dal (Vigna mungo) seed extract 2. Normal blood and Urad dal (Vigna mungo) seed extract
3. PCOS blood and Urad dal (Vigna mungo) seed coat extract
4. Normal blood and Urad dal (Vigna mungo) seed coat extract

The percentage of hemolysis inhibition is calculated in 200µl of the blood sample in presence of extract with volumes ranging from 40µl, 80µl, 120µl, 160µl, 200µl, and 240µl with the concentration of 200µg, 400µg, 600µg, 800µg, 1000µg, 1200µg by measuring the optical density of the samples prepared. The percentage of hemolysis inhibition is calculated with the help of the optical density value obtained from the sample and the control using the Spectrophotometer. The formula used to calculate the percentage of hemolysis inhibition is

$$\% \text{ Of inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Table 1: Parameters required for Anti-inflammatory activity

Parameters	U1	U2	U3	U4	U5	U6	S	C
Concentration in µg/ml	200	400	600	800	1000	1200	_	_
Extract in µg/ml	40	80	120	160	200	240	_	_
Standard in µl	_	_	_	_	_	_	20	_
Control in µl	_	_	_	_	_	_	_	20
Buffer solution (ml)	2	2	2	2	2	2	2	2
Blood (µl)								
Incubate in the water bath for 30 minutes at 56° C and cool in tap water								
Centrifuge for 5 minutes at 2500rpm								
The absorbance of the supernatant at 560nm								

Plate 6: Control and PCOS blood samples with seed and seed coat extract

RESULTS

Percentage of hemolysis inhibition:

The percentage of hemolysis inhibition in the samples containing the mixture of Control blood and Vigna mungo Seed extract:

The percentage of hemolysis inhibition in the unknown sample I is 7%. The percentage of hemolysis inhibition in the unknown sample II is 7%. The percentage of hemolysis inhibition in the unknown sample III is 23%. The percentage of hemolysis inhibition in the unknown sample IV is 38%. The percentage of hemolysis inhibition in the unknown sample V is 53%. The percentage of hemolysis inhibition in the unknown sample VI is 69.2%.

The percentage of hemolysis inhibition in the samples containing the mixture of PCOS blood and Vigna mungo Seed extract:

The percentage of hemolysis inhibition in the unknown sample I is 0%. The percentage of hemolysis inhibition in unknown sample II is 12.5%. The percentage of hemolysis inhibition in the unknown sample III is 37.5%. The percentage of hemolysis inhibition in the unknown sample IV is 50%. The percentage of hemolysis inhibition in the unknown sample V is 62.5%. The percentage of hemolysis inhibition in the unknown sample VI is 87.5%.

The percentage of hemolysis inhibition in the samples containing the mixture of Control blood and Vigna mungo Seed coat extract:

The percentage of hemolysis inhibition in the unknown sample I is 0%. The percentage of hemolysis inhibition in the unknown sample II is 25%. The percentage of hemolysis inhibition in the unknown sample III is 50%. The percentage of hemolysis inhibition in the unknown sample IV is 75%. The percentage of hemolysis inhibition in the unknown sample V is 83%. The percentage of hemolysis inhibition in the unknown sample VI is 91.6%.

The percentage of hemolysis inhibition in the samples containing the mixture of PCOS blood and Vigna mungo Seed coat extract.

The percentage of hemolysis inhibition in the unknown sample I is 62.5%. The percentage of hemolysis inhibition in the unknown sample II is 25%. The percentage of hemolysis inhibition in the unknown sample III is %. The percentage of hemolysis inhibition in the unknown sample IV is 37.5%. The percentage of

hemolysis inhibition in the unknown sample V is 87.5%. The percentage of hemolysis inhibition in the unknown sample VI is 50%.

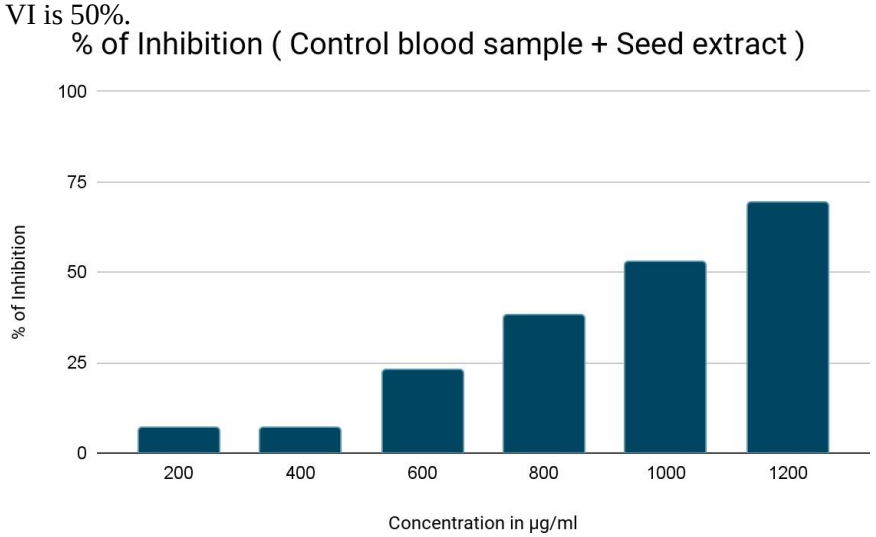

Fig1: Percentage of Inhibition in the samples with Control blood and seed extract

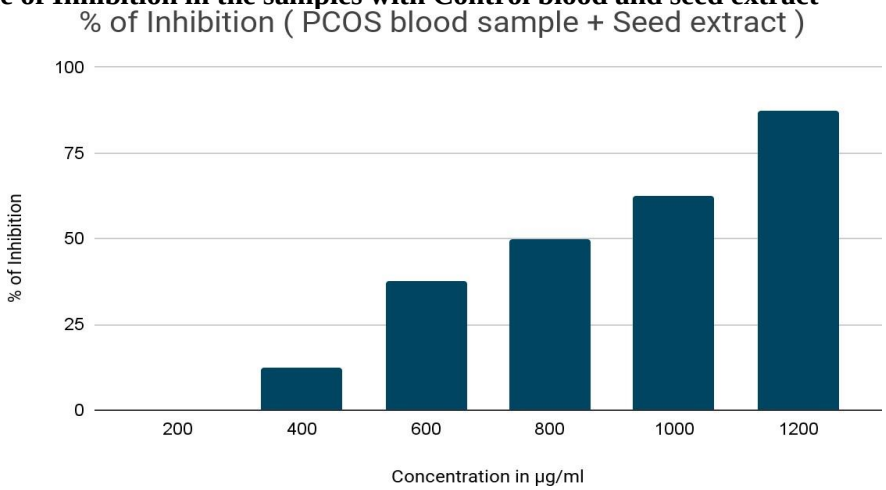

Fig 2: Percentage of Inhibition in the samples with PCOS blood and seed extract

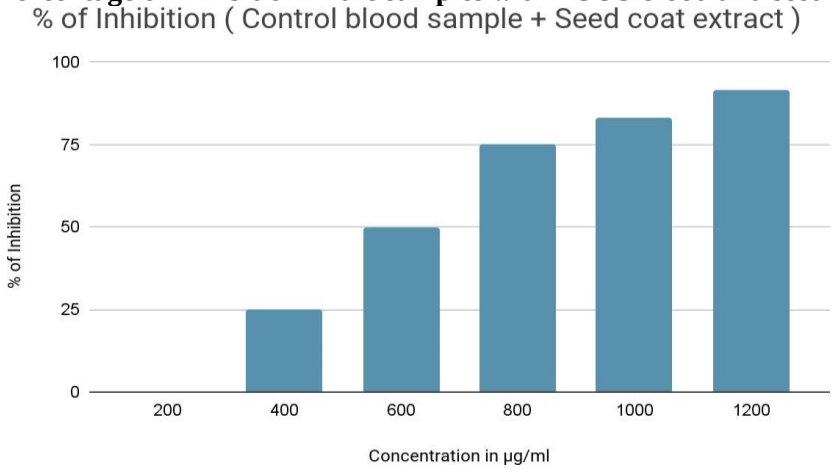

Fig 3: Percentage of Inhibition in the samples with control blood and seed coat extract

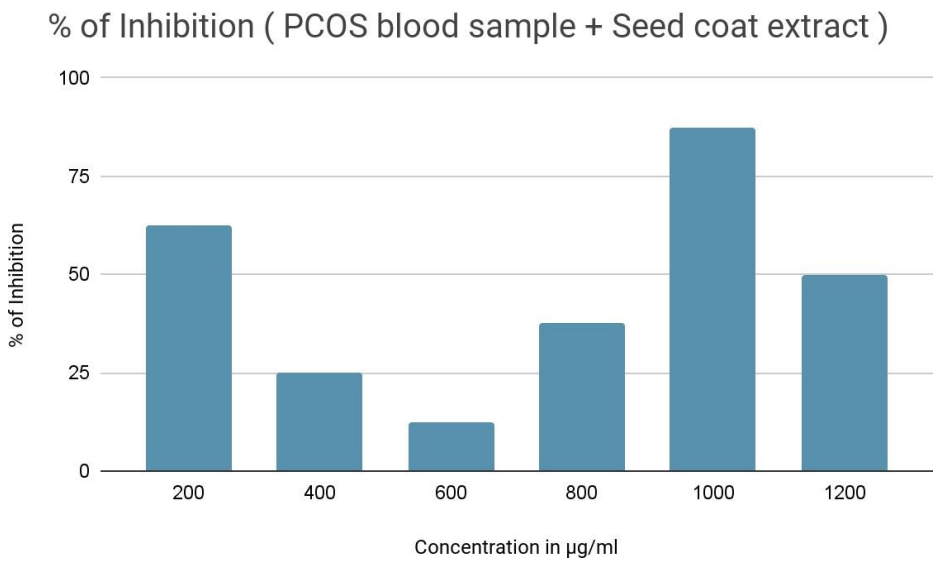

Fig 4: Percentage of Inhibition in the samples with PCOS blood and seed coat extract

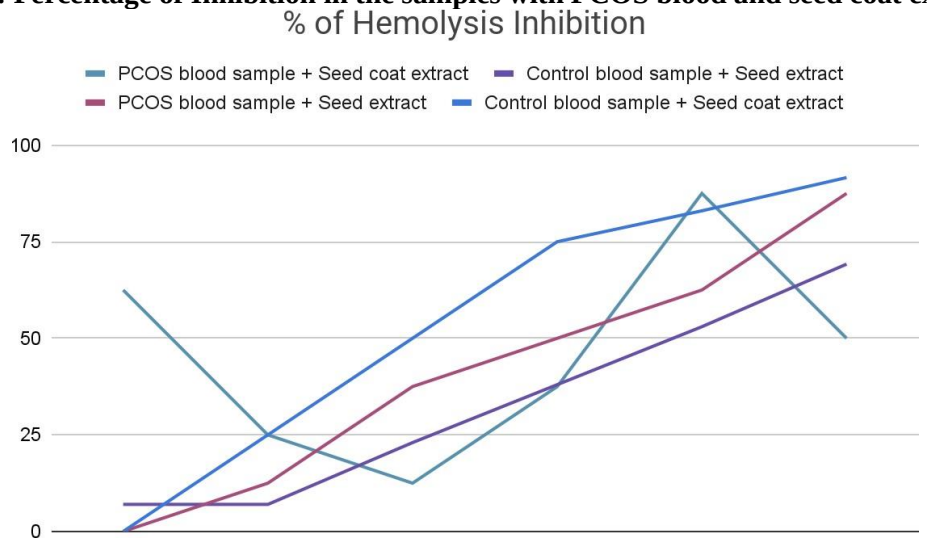

Fig 5: Comparison of the percentage of hemolysis inhibition in all the samples

DISCUSSION

On account of the critical role played by leukocytes, cellular infiltration is a crucial feature of an inflammatory response. Leukocytes release lysosomal enzymes, including proteases, as part of their protective activities during inflammation, causing additional tissue damage and inflammation. Damage to cell membranes increases the cell's vulnerability to subsequent damage caused by free radical-induced lipid peroxidation. Membrane proteins regulate the volume and water content of cells by directing the passage of sodium and potassium ions, and injury to the membrane affects this function (Gunathilake, 2018).

Because the membranes of red blood cells and lysosomes are similar, inhibiting red blood cell hemolysis may provide insight into the inflammatory process. The lysis and subsequent release of the cytoplasmic contents may be delayed or inhibited by stabilizing these cell membranes, which reduces tissue damage and, hence, the inflammatory response. As a result, chemicals that contribute to significant cell membrane protection against harmful compounds are vital in the prevention of inflammation.

Supplementation of black gram (*Vigna mungo)*, both with and without skin, has a proinflammatory and prooxidant effect, providing scientific support for the control of black gram in an arthritic diet.

The activities of inflammatory mediators such as lipoxygenase, myeloperoxidase, nitric oxide synthase, and Peripheral blood mononuclear cells are elevated upon having a diet that mainly contains *Vigna mungo* with and without seed coat (Helen Antony, 2016).

Upon analysis, of the samples containing seed extract the percentage of hemolysis inhibition is higher than 50% at a maximum concentration which supports the fact black gram inhibits the destruction of RBCs by anti-inflammatory effects.

By analyzing the samples containing seed coat extract the percentage of hemolysis inhibition is higher than 50% at a

maximum concentration which supports the fact that the seed coat of black gram inhibits the destruction of RBCs by anti-inflammatory effects.

Analysis of percentage of inhibition in the samples used:

SEED EXTRACT:

CONTROL BLOOD:

The Optical density of the Control blood i.e., the blood of a person without PCOS, and the *Vigna mungo* seed extract were read at 560nm using a Spectrophotometer. From the Optical density value obtained from the six different samples with different concentrations of extract, it is concluded that Sample VI with the extract concentration of 1200µg has the highest value of hemolysis inhibition which is 69.2%.

PCOS BLOOD:

The Optical density of the PCOS blood and the *Vigna mungo* seed extract was read at 560nm using Spectrophotometer. From the Optical density value obtained from the six different samples with different concentrations of extract, it is concluded that Sample VI with the extract concentration of 1200µg has the highest value of hemolysis inhibition which is 87.5%.

The percentage of hemolysis inhibition in the PCOS blood is Comparatively higher than it is in the control blood. This is due to the enhancement of the enzymes which gives the Anti-inflammatory effect. This was bought by *Vigna mungo* extract present in the sample which prevents the destruction of RBCs.

SEED COAT EXTRACT:

CONTROL BLOOD:

The Optical density of the Control blood i.e., the blood of a person without PCOS, and the *Vigna mungo* seed coat extract was read at 560nm using Spectrophotometer.

From the Optical density value obtained from the six different samples with different concentrations of extract, it is concluded that Sample VI with the extract concentration of 1200µg has the highest value of hemolysis inhibition which is 91.6%.

PCOS BLOOD:

The Optical density of the PCOS blood and the *Vigna mungo* seed extract was read at 560nm using Spectrophotometer. From the Optical density value obtained from the six different samples with different concentrations of extract, it is concluded that Sample V with the extract concentration of 1000µg has the highest value of hemolysis inhibition which is 87.5%.

In this case, the percentage of hemolysis inhibition in the PCOS blood is Comparatively lower than it is in the control blood. By the results obtained it can be said that the anti-inflammatory effect, the level of hemolysis inhibition is higher in the presence of seed coat extract than in the presence of seed extract.

CONCLUSION

From the experiment, it is concluded that the hemolysis inhibition is more in the PCOS blood sample than in the Control blood sample in the presence of the seed extract. This is due to the anti-inflammatory effect of the *Vigna mungo* which prevents the destruction of RBCs.

From the experiment, it is concluded that the hemolysis inhibition is comparatively less in the PCOS blood sample than in the Control blood sample in the presence of the *Vigna mungo* seed coat extract.

Comparing the values obtained from both the *Vigna mungo* seed extract and the *Vigna mungo* seed coat. it is clear that the *Vigna mungo* seed coat shows a higher hemolysis inhibition than the seed.

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