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Standardization, Phytochemical and *Invivo* Anti-Diabetic Screening of *Aragwadhadi kashayam* by using Wistar Albino rats.

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Abstract : Diabetic mellitus describes metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, lipid, protein metabolism resulting defects in inslin secretion, insulin action or both and an increased risk of complications from vascular diseases . The burden of diabetes is high and increasing globally and in developing economies like India, mainly fueled by the increasing prevalence of overweight/obesity and unhealthy lifestyles. The estimates in 2019 showed that 77 million individuals had diabetes in India, which is expected to rise to over 134 million by 2045. Hypoglycemia and weight gain are seen frequently in patients undergoing diabetic treatment which also decreases the treatment adherence. The constant loss of endogenous islet cell reserve is the main reason for the need of intensified therapies. Current treatments have not fully improved long-term beta cell function. It is desirable to move forward to obtain new drugs that offer solutions sustainable in the long term and should be able to fit the individual circumstances and preferences of patients with diabetes mellitus.The aim of this study is the standardization of the Ayurvedic drug *Aragwadhadi kashayam* and to evaluate the *invivo* anti-diabetic activity of *Aragwadhadi kashayam* by using wistar albino rats.

Keywords: Diabetic mellitus, sustainable solutions, *invivo* anti-diabetic activity, *Aragwadhadi kashayam.*

I.INTRODUCTION

Diabetic mellitus describes metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, lipid, protein metabolism resulting defects in inslin secretion, insulin action or both and an increased risk of complications from vascular disease. Diabetic mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss. In its most severe forms ketoacidosis and non-ketotic hyperosmolar state may develop and leads to stupor and coma and in absence of effective treatment death. The longterm effects of diabetes mellitus include progressive development of specific complications of retinopathy with potential blindness, nephropathy that may leads to renal failure, and neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfuntion, includes sexual dysfuntion. People with diabetes are at increased cardiovascular, peripheral vascular and cerebrovascular disease.[1] Hypoglycemia and weight gain are seen frequently in patients undergoing diabetic treatment which also decreases the treatment adherence. The constant loss of endogenous islet cell reserve is the main reason for the need of intensified therapies. Current treatments have not fully improved long-term beta cell function. It is desirable to move forward to obtain new drugs that offer solutions sustainable in the long term. These drugs should be able to fit the individual circumstances and preferences of patients with diabetes mellitus. The aim of this study is the standardization of the ayurvedic drug *Aragwadhadi kashayam* and to evaluate the *in vivo* anti-diabetic activity of *Aragwadhadi kashayam* by using wistar albino rats. The main objectives of the study are standardization of the *Aragwadhadi kashayam* using methods like phytochemical, physiochemicals and microbial testing, evaluation of anti-diabetic activity of *Aragwadhadi kashayam* using *in vivo* study includes the chemically induced anti-diabetic activity, the present study is an attempt to investigate the effect of *Aragwadhadi kashayam* on Alloxan induced diabetes in wistar albino rats.

II.MATERIALS AND METHODS

II.1 ANIMALS

Healthy, adult Wistar albino rats of both sexes (150-220g) were obtained from the animal house facility R.V.S College of Pharmaceutical Sciences, sulur, Coimbatore, Tamilnadu. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20 ± 3ºC. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by Animal Ethical Committee of Department Pharmacology, R.V.S. College of Pharmaceutical Sciences, Coimbatore (approval no: 1012/c/06/CPCSEA).

II.2 PHYTOCHEMICAL SCREENING

II.2.1 Qualitative phytochemical screening

Standard screening tests were carried out for various plant constituents. The crude samples were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures.

1.Test for alkaloids

a. Preliminary test

A 100 mg of an sample was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendroff 's and Mayer's reagents. The treated solutions were observed for any precipitation.

b. Confirmatory test

Five grams of the sample was treated with 40% calcium hydroxide solution until the sample was distinctly alkaline to litmus paper, and then sampleed twice with 10 ml portions of chloroform. Chloroform samples were combined and concentrated in vacuo to about 5 ml. Chloroform sample was then spotted on thin layer plates. Solvent system (n-hexaneethyl acetate, 4:1) was used to develop chromatograms and detected by spraying the chromatograms with freshly prepared Dragendroff 's spray reagent. An orange or dark colored spots against a paleyellow background was confirmatory evidence for the presence of alkaloids.

c. Dragendorff's test

To the exract, add 1ml of Dragendorff's reagent. An orange red colored precipitate indicates the presence of alkaloids.

d. Wagner's test

To the sample, add 1ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

e. Mayer's test

To the sample, add 1ml of Mayer's reagent. A dull white colored precipitate indicates the presence of alkaloids.

f. Hager's test

To the sample, add 3ml of Hager's reagent. Yellow colored precipitate indicates the presence of alkaloids.

2. Tests for carbohydrates

Molish test

To the sample, 1ml of α- naphthol solution was added and conc. Sulphuric acid was added along with the sides of the test tube. Purple or reddish violet color at the unction between the two liquids indicates the presence of carbohydrates.

Fehling test

To the sample, equal quantities of Fehling's solution A & B were added. Upon heating gently, a rick red precipitate indicates the presence of carohydrates.

Benedict's test

To 5ml of Benedict's reagent, 8 drops of solution under the test was added and mixed well. Then it was boiled vigorously for 2 minutes and cooled. Red precipitate indicates the presence of carbohydrates.

3.Tests for proteins

Biuret test

To the sample, 1ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution was added. A violet color indicates the presence of proteins.

Lead Acetate test

To the sample, 1ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

4.Test for Steroidal Compounds

a. Salkowski's test

0.5 g sample were dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e., the aglycone portion of the glycoside).

b. Lieberman's test

0.5 g sample were dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added. A color change from purple to blue to green indicated the presence of a steroid nucleus, i.e., aglycone portion of the cardiac glycosides.

5. Tests for amino acids

Xanthoprotic test

To the sample, 1ml of conc. Nitric acid was added. When a white precipitate was formed, it is boiled and cooled. Orange color indicates the presence of aromatic amino acids.

Ninhydrin test

2 drops of freshly prepared 0.2% ninhydrine reagent was added to the sample and heated. Development of blue color indicates the presence of proteins, peptides or amino acids.

6. Test for phenolic compounds

The sample were dissolved in water. Few crystals of ferric sulfate were added to the mixture. Formation of dark-violet color indicated the presence of phenolic compounds.

7. Tests for Flavonoids

a. Test for free flavonoids

Five milliliters of ethyl acetate was added to a solution of 0.5 g of the sample in water. The mixture was shaken, allowed to settle, and inspected for the production of yellow color in the organic layer, which is taken as positive for free flavonoids.

b. Lead acetate test

To a solution of 0.5 g sample in water about 1 ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

c.Reaction with sodium hydroxide

Dilute sodium hydroxide solution was added to a solution of 0.5 g of the sample in water. The mixture was inspected for the production of yellow color which considered as positive test for flavonoids.

8. Test for saponins

Froth test

0.5 g sample were dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 sec. The test tube was allowed to stand in a vertical position and observed over a 30-min period of time. If a "honey comb" froth above the surface of liquid persists after 30 min. the sample is suspected to contain saponins.

Foam test

About 1ml of sample is diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins.

9. Test for tannins

a. Ferric chloride test

A portion of the sample was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

b. Formaldehyde test

To a solution of about 0.5 g sample in 5 ml water, three drops of formaldehyde and six drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1 min and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol, and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a colored residue after washing, indicated the presence of phlobatannins.

c. Test for phlobatannins

Deposition of a red precipitate when an aqueous sample of the plant part was boiled with 1% aqueous hydrochloric acid was taken as an evidence for the presence of phlobatannins. d. Modified iron complex test. To a solution of 0.5 g of the plant sample in 5 milliliter of water a drop of 33% acetic acid and 1 g sodium potassium tartarate was added. The mixture was warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of color is obtained and then boiled. Purple or blackish precipitates, which are insoluble in hot water, alcohol, or dilute ammonia, denote the presence of pyrogallol tannin.

10. Test for anthraquinones

a. Test for free anthraquinones (Borntrager's test)

The hydrosamples of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered, and 5 ml of 10% ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free anthraquinones.

b. Test for O-anthraquinone glycosides (Modified Borntrager's test)

For combined anthraquinones, 5 g plant samples were boiled with 10 ml 5% sulfuric acid for 1 h and filtered while hot. The filtrate was shaken with 5 ml benzene; the benzene layer separated and half its own volume of 10% ammonia solution added. The formation of a pink, red, or violet color in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the sample.

11. Tests for cardiac glycosides

a.Keller – killani test

Test sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. Sulphuric acid. At the junction, reddish brown color was formed, which gradually becomes blue indicates the presence of cardiac glycosides.

II.2.2 Physiochemical studies

A.Organoleptic properties

This formulation was evaluated for its organoleptic properties viz. odour, appearance and taste.

B.PH

The PH was determined using a calibrated PH meter.

C. Specific gravity

The specific gravity of formlation was determined using 10 ml specific gravity bottles and calculated using the following formula

$$\text{Specific gravity of liquid (formlation)} = \frac{\text{Weight of 10 ml of liquid / 10}}{\text{Weight of 10 ml of water / 10}}$$

D. Total solid content

This was determined by taking 10 ml of the formulation in porcelain evaporating dish and heating it on an electric water bath at 60 -70º and then in an oven at 105º C until constant weight of residue was obtained.

E. Microbial test

This involved determination of total bacterial count and total fungal count. Each of the microbial experiments was performed in a sterilized laminar air flow (LAF) chamber. This experiment was performed using a sample from newly opened bottle. The sample stock solution was prepared by taking 10 ml of formulation and making up the volume to 100 ml with Soyabean Casein Digest (SCD) Broth. 1 ml of this stock solution was used as test sample for all the microbial tests. Four control petri plates viz. medium control, diluent (negative) control, positive control (bacterial and fungus culture) and environment control were set for each experiment. For total bacterial count, the test samples along with control samples were incubated in soyabean Casein Digest Agar (SCDM) medium at 35º C for 2-3 days. For total fungal count, the test samples were incubated in Sabouraud Dextrose Agar (SDA) medium at 25ºC for 5-7 days.[3]

II.3 PHARMACOLOGICAL STUDIES

II.3.1 *Invivo* antidiabetic activity of *Aragwadhadi kashayam* in alloxan induced diabetic wistar albino rats:

II.3.1.1 Induction of diabetes in rats:

Diabetes was induced by single intraperitoneal administration of alloxan monohydrate in rats (120 mg/kg). The blood samples were collected from retro-orbital plexus on 21th day and blood glucose levels were estimated. Rats having blood glucose levels above 200 mg/dl were selected for further experiments and divided in five groups of six rats each. All groups were treated orally once a day for 21 days. The blood glucose

levels were evaluated at regular time intervals at 0, 2, 4 and 24 h after the first treatment (acute treatment) and on the seventh day 1 h after the last treatment (chronic treatment).[3]

II.3.1.2 Experimental design:

Diabetic and normal rats were randomly divided into five groups of rats were employed in the present study and each group contains six animals, as follows

Group I: Normal control (saline).
Group II: Alloxan treated control (120 mg/kg. i.p.).
Group III: Alloxan (120 mg/kg.i.p...) + Standard drug, Glibenclamide (5 mg/kg, p.o).
Group IV: Alloxan (120 mg/kg. i.p) + Aragwadhadi kashayam (200 mg/kg, p.o),
Group V: Alloxan (120 mg/kg.i.p) + Aragwadhadi kashayam (400 mg/kg, p.o),

Doses of Aragwadhadi kashayam were determined according to a preliminary experiment. Aragwadhadi kashayam, saline and glibenclamide were orally administrated by gavage once a day for 21 consecutive days. Fasting blood glucose level and body weight were determined on days of the 2nd, 4th, 7th, 10th, 14th and 21th after the Aragwadhadi kashayam administration. On the 21th day, animals of all groups were anaesthetized by giving an intraperitoneal injection of 40 mg/kg pentobarbital sodium and blood serum of each rat was collected.

Group I serve as normal control which received normal saline for 21 days. Group II were diabetic control rats. Group IV and Group V (which previously received Alloxan 120 mg/kg) were given fixed doses of Aragwadhadi kashayam (200mg/kg, 400 mg/kg p.o,) and Group III received standared drug Glibenclamide (5 mg/kg p.o) for 21 consective days.[4]

II.3.1.3 Collection of blood samples:

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, 14 and 21 days.

II.3.1.4 Estimation of biochemical parameters serum blood glucose:

On 1, 7, 14 and 21days fasting blood samples were collected and analysed the blood glucose.

II.3.1.4.1 Blood glucose level:

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

II.3.1.4.2 Determination of haemoglobin content

The haemoglobin tube (STD 14.5gm = 100% concentration) was filled with N/10 hydrochloric acid (HCL) upto 2 gm marking. This graduated tube was placed in sahli's Haemoglobinometer (Comparator with Brown glass). Blood sample obtained from capillary or venous blood was drawn in Sahli's pipette up to 20µl mark and added in haemoglobin tube containing N/10 HCL. The blood and acid are mixed with glass stirrer and allowed to stand for 5 minutes for acid haematin formation. Drop by drop distilled water was added to dilute the acid haematin compound colour till it matches with the standard colour plates of the comparator. Results were read as gms/dl present on the haemoglobin tube.[5]

II.3.1.4.3 Determination of kidney functions

Estimation of Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated level of urea in blood are referred to as hyper uremia or azotemia.Urease- Glutamate dehydrogenase (GLDH): enzymatic UV test (64)

Procedure

- ✓ Taken 1000µl of reagent-1 and 250µl of reagent-2 in 5ml test tube.
- ✓ To this added 10µl of serum
- ✓ Mixed well and immediately read the test sample at 340nm Hg 334nm Hg 365nm
- ✓ optical path 1 cm reagent blank (2-point kinetic)
- ✓ Normal range: 10-50mg/dl [2]

Estimation of Creatinine

Estimation of Creatinine by Jaffe Method (modified). Creatinine forms a coloured complex with picrate in alkaline medium.The rate of formation of the complex is measured.

Procedure

- ✓ Take 500µl of reagent-2 and 500µl of reagent-3 in a 5ml test tube
- ✓ To this add 100µl of serum
- ✓ Mix well and immediately read the test sample at Hg 492nm 1cm light path and note
- ✓ Downs the values.
- ✓ Normal range is 0.6-1.1mg/dl

II.3.1.4.4 Determination of Total Bilirubin

Bilirubin reacts with diazotized sulphanilic acid to form an azobilirubin compound the colour of which is measured at 546 nm (530-560 nm) and is proportional to the concentration of bilirubin. For total bilirubin the reaction is accelerated by caffeine reagent. The readings for total and direct bilirubin are taken after 2 minutes incubation.

Bilirubin + Diazotized Sulphanilic Acid Azobilirubin Compound

Pipette into clean dry test tubes labelled as Blank (B) and Test (T) add a ml Direct bilirubin reagent in both the test tubes and then add 0.05 ml Direct nitrite reagent to the test (T) test tube and add 0.1 ml sample in both test tubes. Mix well and incubate at room temperature for exactly 5 minutes. Measure the absorbance (546 nm) of the test Samples (Abs. T) immediately against their respective Blanks. The result was calculated by using the following formula[6]

Total or Direct bilirubin (mg/dl) = Abs. T X Abs. S

II.3.1.4.5 Determination of Alkaline phosphatase (ALP)

Alkaline Phosphatase in serum catalyse the hydrolysis of p. nitrophenyl phosphate to p- nitrophenol and phosphate. p. nitrophenol is yellow coloured compound. As the reaction progresses the rate of absorbance increases which is proportional to the activity of Alkaline Phosphatase in the sample. This reaction takes place in alkaline medium and in presence of magnesium ions. The change in absorbance is measured at 405 nm.[7]

II.3.1.4.6 Estimation of Aspartate Aminotransferase (AST)

The AST was estimated by using the kit obtained from Span Diagnostics. Modified UV (IFCC), Kinetic Assay. Aspartate aminotransferase (AST) can catalyses the transamination of L-Aspartate and α-ketoglutarate to appeared L-Glutamate and oxaloacetate. Malate Dehydrogenase reduces Oxaloacetate to Malate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide to Nicotinamide Adenine Dinucleotide. Sum of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and it is proportional to AST commodity sample.[75] Mix well and aspirated immediately for measurement. Analyzer was programmed as per assay parameters. Analyzer was blank with purified water. Absorbance was read after 60 seconds. Reading was repeated after every 30 seconds i.e. upto 120 seconds at 340 nm wavelength. Mean absorbance changes per minute (ΔA / minute) were determined.

AST activity was calculated by using the formula:

AST activity (IU/L) = ΔA / minute X kinetic factor

ΔA / minute - change in absorbance per minute.

Kinetic factor (K) = 1768.

II.3.1.4.7 Estimation of Alanine aminotransferase (ALT)

The ALT was estimated by using the kit obtained from Span Diagnostics. Modified UV (IFCC), Kinetic Assay. Alanine aminotransferase can catalyses the transamination of L-Alanine and α ketoglutarate to get pyruvate and L-Glutamate.[7] Lactate dehydrogenase (LD) reduces pyruvate to Lactate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide to Nicotinamide Adenine Dinucleotide. The rate of oxidation of NADH is measured kinetically by monitoring the condense in 340 nm of absorbance and is straight comparative to ALT activity of sample. Mixed well and aspirated immediately for measurement. Analyzer was programmed as per assay parameters. Analyzer was blank with purified water. Absorbance was read after 60 seconds. Reading was repeated after every 30 seconds i.e. up to 120 seconds at 340 nm wavelength. Mean absorbance changes per minute (ΔA / minute) were determined. AST activity was calculated by using the formula

ALT activity (IU/L) = ΔA / minute X kinetic factor

ΔA / minute - change in absorbance per minute.

Kinetic factor (K) = 1768.

II.4 STATISTICAL ANALYSIS:

The determination of Percentage Inhibition and IC50 Value. In animal study, data were expressed as mean ± SEM. The statistical analysis was carried out using oneway analysis of variance (ANOVA), followed by post hoc analysis Tukey's multiple comparison tests using prism 5.0 and values of $P < 0.05$ were considered as statistically significant.

III RESULT AND DISCUSSION

III.1 Preliminary Phytochemical analysis:

Table: 1 Qualitative phytochemical screening of *Aragwadhadi kashayam*

S.NO	Phytochemicals	Presence/ Absence
1.	Alkaloids • Dragendorff's test • Mayer's test	+
2.	Flavonoids • Lead acetate test • Reaction with sodium	+
3.	Tannins • Ferric test • Formaldehyde test • Test for phlobatannins	+
4.	Carbohydrate • Molish test • Fehling test	–
5.	Phenol	+
6.	Glycosides • Keller- killani test	+
7.	Saponins • Forth test • Foam test	+
8.	Proteins and amino acids test • Biuret test • Ninhydrin test • Xanthoprotic test	+

9.	Steroids and sterols • Salkowski's test • Lieberman's test	+

III.2 Physiochemical analylsis of *Aragwadhadi kashayam*:

The Physiochemical analysis on *Aragwadhadi kashayam* shows the following results:

1.Organoleptic characters of *Aragwadhadi kashayam*

Table: 2 Organoleptic properties of *Aragwadhadi kashayam*

S. No	Organoleptic properties	Observation
1.	State	Liquid
2.	Odour	Characteristic
3.	Touch	Non- greasy
4.	Flow property	Free flowing
5.	Appearance	Brownish

2.pH value

The pH value of the *Aragwadhadi kashayam* is 3.7, which is acidic in nature.

3.Specific Gravity

The specific gravity of the Ayurvedic drug *Aragwadhadi kashayam* was found to be1.02492 (± 0.025).

4.Total solid content

The Total solid content of the Ayurvedic drug *Aragwadhadi kashayam* was found to be21.1977 (± 0.165).

5.Microbial test

The microbial testing was done for the *Aragwadhadi kashayam* using cotton swab method. The results obtained were in compliance with the protocol for testing Ayurveda, Siddhaand Unani medicines i.e.1×105 CFU/ml for total bacterial count and 1×103 CFU/ml for total fungal count.

III.3 Effect of *Aragwadhadi kashayam* on food intake

Table: 3 Effect of *Aragwadhadi kashayam* on food intake in diabetic rats

S.No	Groups	1^{st}day grams	14^{th}day grams	21^{st}day grams
1.	Control	26.3 ± 2.1	25.5 ± 2.5	27.2 ± 2.1
2.	Alloxan (120 mg/kg. i.p.)	27.3 ± 2.4	26.0 ± 2.1	29.6 ± 2.9
3.	Glibenclamide(5mg/kg, p.o)	26.1 ± 3.1	25.7 ± 2.9	26.2 ± 3.0
4.	*Aragwadhadi kashayam* (200mg/kg)	26.6 ± 2.8	27.0 ± 3.0	27.2 ± 3.0
5.	*Aragwadhadi kashayam* (400mg/kg)	27.3 ± 2.5	28.0 ± 2.9	28.6 ± 2.5

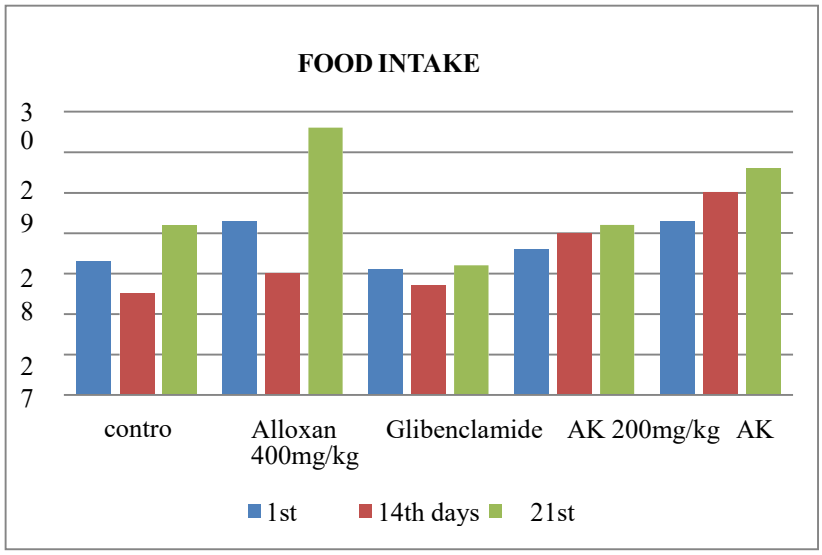


Fig 1: Effect of *Aragwadhadi kashayam* on food intake.

III.4 Effect of *Aragwadhadi kashayam* on water intake.

Table: 4 Effect of *Aragwadhadi kashayam* on water intake in diabetic rats

S.No	Groups	1st day (ml)	14th day (ml)	21th day (ml)
1.	Control	8.5 ± 1.5	8.4 ± 1.6	8.5 ± 2.2
2.	Alloxan (120 mg/kg. i.p.)	7.9 ± 1.5	9.8 ± 2.1**	16.5 ± 1.5***
3.	Glibenclamide (5 mg/kg p.o.)	7.5 ± 2.3	8.5 ± 2.5	9.0 ± 2.1*
4.	*Aragwadhadi kashayam* 200mg/kg	8.0 ± 2.1	8.5 ± 2.3	9.5 ± 2.3*
5.	*Aragwadhadi kashayam* 400mg/kg	8.3 ± 1.6	9.0 ± 1.3	11.5 ± 2.1*

III.5 Effect of *Aragwadhadi kashayam* on body weight.

Table: 5 Effect of *Aragwadhadi kashayam* on body weight in diabetic rats

S.No	Groups	1st day (gms)	14th day (gms)	21st day (gms)
1.	Control	169 ± 20	175 ± 13	191 ± 12
2.	Alloxan(120 mg/kg i.p)	171 ± 15	213 ± 10	245 ± 14
3.	Glibenclamide (5mg/kg p.o.)	179 ± 13	191 ± 12	211 ± 13*
4.	*Aragwadhadi kashayam* (200 mg/kg)	168 ± 12	211 ± 10	198 ± 10*
5.	*Aragwadhadi kashayam* (400 mg/kg)	156 ± 10	203 ± 12	207 ± 17*

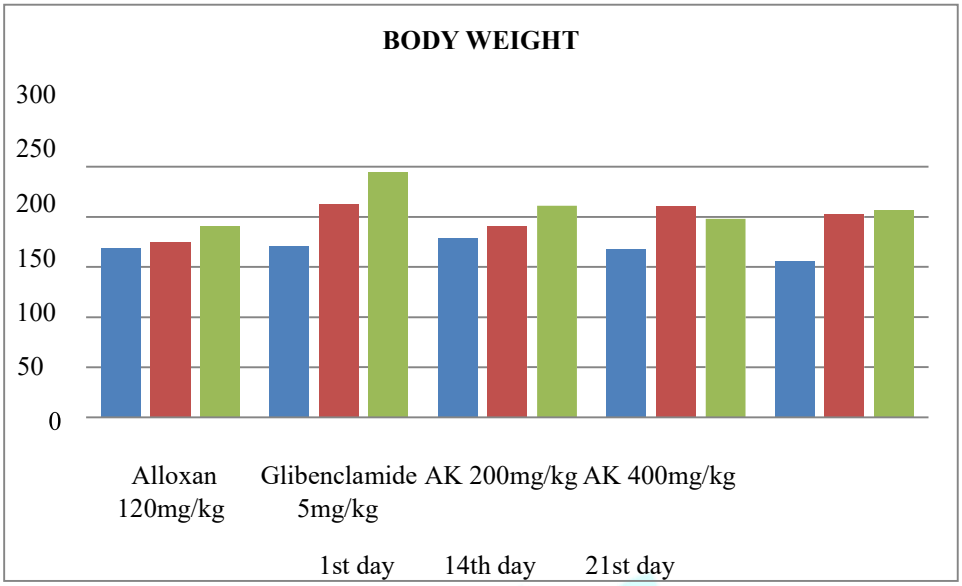


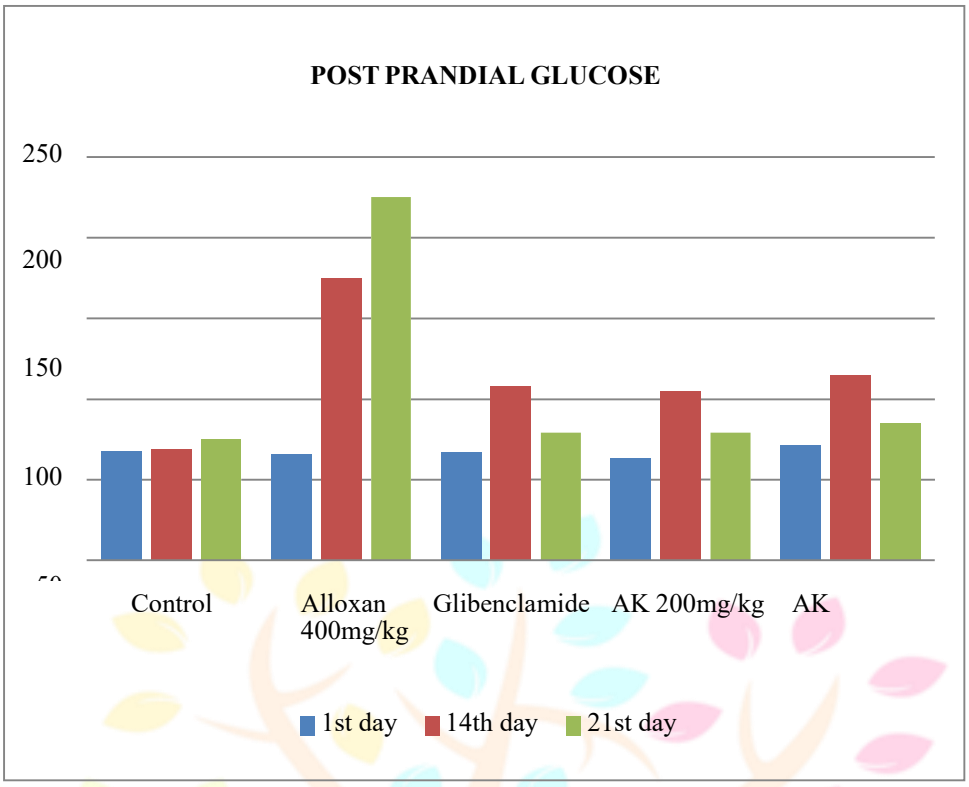
Fig 2: Effect of *Aragwadhadi kashayam* on body weight.

III.6 Effect of *Aragwadhadi kashayam* on postprandial glucose levels

Table: 6 Effect of *Aragwadhadi kashayam* on Post prandial glucose in diabetic rats

S .No	Groups	1st day mg/dl	14th day mg/kg	21st day mg/kg
1.	Control	68 ± 5.0	69 ± 4.5	75 ± 4.8
2.	Alloxan (120mg/kg)	66 ± 7	175 ± 7	225 ± 6
3.	Glibenclamide (5mg/kg p.o)	67 ± 8	108 ± 7*	79 ± 9*
4.	*Aragwadhadi kashayam* (200mg/kg)	63 ± 5	105 ± 8*	79 ± 7*
5.	*Aragwadhadi kashayam* (400mg/kg)	71 ±8	115 ± 6*	85 ± 7*

Figure 3: Effect of *Aragwadhadi kashayam* on postprandial glucose levels



III.7 Effect of *Aragwadhadi kashayam* on haemoglobin:

Table: 7 Effect of *Aragwadhadi kashayam* on Haemoglobin in diabetic rats

Groups	1st day (g/dL)	21st day (g/dL)
Control	13.9 ± 1.3	13.8 ± 1.5
Alloxan (120 mg/kg.i.p)	14.2 ± 1.5	6.9 ± 1.8
Glibenclamide (5 mg/kg,p.o)	15.1 ± 1.7	12.9 ± 1.5***
Aragwadhadi kashayam 200mg/kg	13.2 ± 1.8	11.9 ± 1.8***
Aragwadhadi kashayam 400mg/kg	13.7 ± 2.0	12.5 ± 1.6***

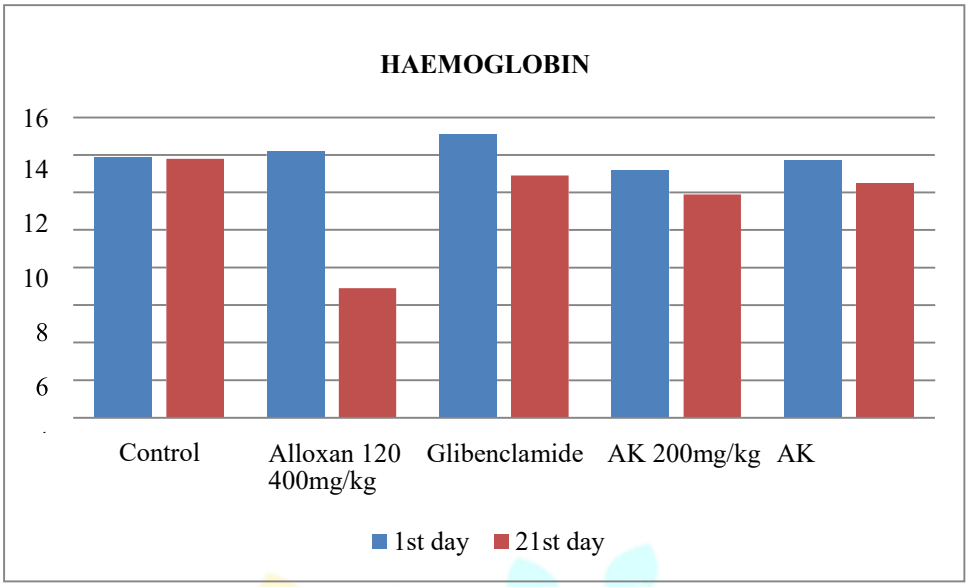


Figure 4: Effect of *Aragwadhadi kashayam* on haemoglobin.

III.8 Effect of *Aragwadhadi kashayam* on liver function test:

Table:8 Effect of *Aragwadhadi kashayam* on Liver function test in diabetic rats

Groups	AST (U/L)	ALT (U/L)	ALP(U/L)
Control	64.33 ± 2.94	33.5 ± 2.37	74.5 ± 2.17
Alloxan (120mg/kg i.p.)	79 ± 3.03	49.33 ± 2.16	100 ± 2.82
Glibenclamide (5mg/kg p.o.)	69.67 ± 2.94**	35 ± 2.37**	81 ± 2.61**
Aragwadhadi kashayam (200mg/kg)	72.83 ± 2.23*	45.5 ± 1.87*	85.17 ± 4.71*
Aragwadhadi kashayam (400mg/kg)	72.83 ± 2.14*	42.33 ± 2.16*	84.24 ± 2.97*

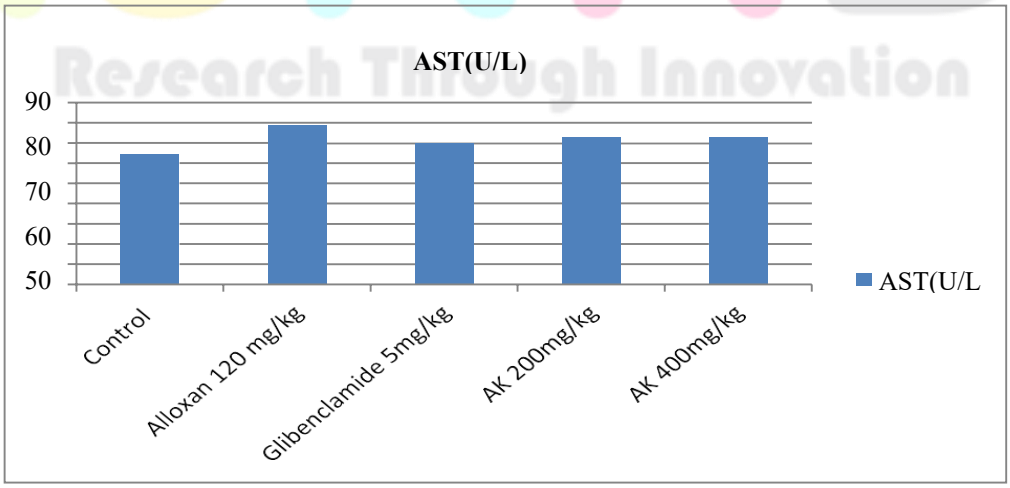


Fig 5: Effect of *Aragwadhadi kashayam* on AST.

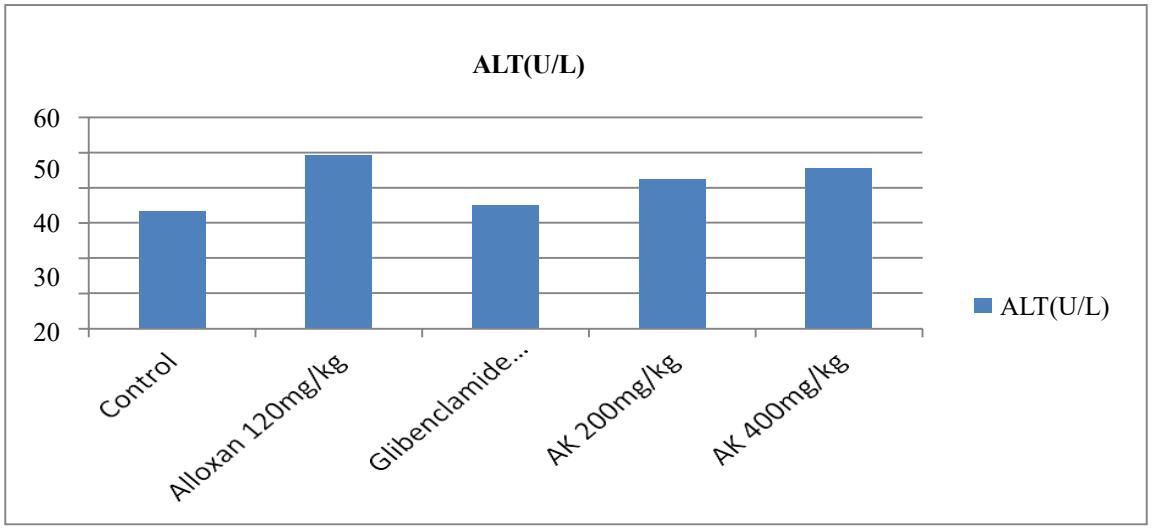


Fig 6: Effect of *Aragwadhadi kashayam* on ALT.

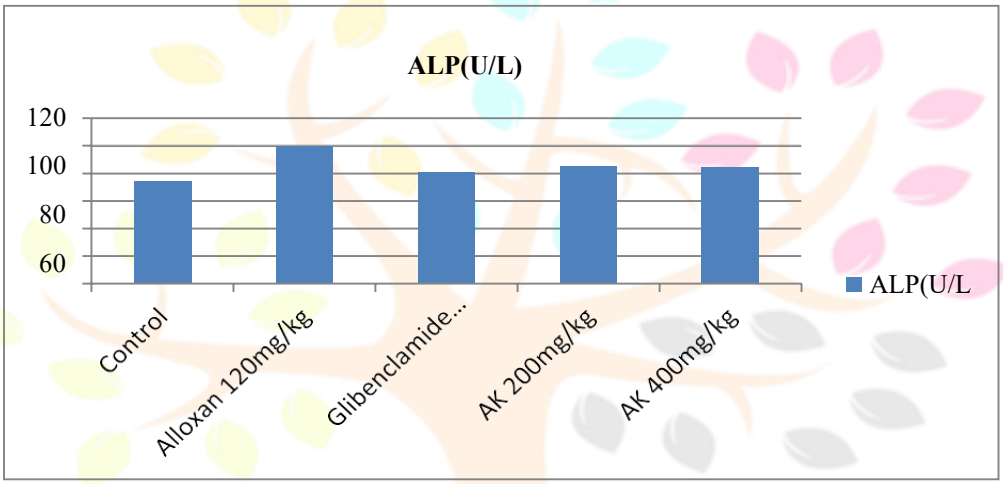


Fig 7: Effect of *Aragwadhadi kashaym* on ALP.

III.9 Effect of *Aragwadhadi kasha*yam on blood urea, creatinine and bilirubin

Table:9 Effect of *Aragwadhadi kashayam* on blood urea, creatinine and bilirubin in diabetic rats

Groups	CREATININE (µmol/L)	UREA (mmol/l)	BILIRUBIN (µmol/L)
Control	37.33 ± 2.5	1.21 ± 0.13	8.24 ± 0.28
Alloxan (120mg/kg.i.p)	53.83 ± 2.86	6.05 ± 0.26	13.57 ± 0.35
Glibenclamide(5mg/kg,p.o)	41.33 ± 1.63**	1.45 ± 0.15***	8.95 ± 0.25***
Aragwadhadi kashayam (200mg/kg)	44.33 ± 3.08*	2.15 ± 0.11***	10.21 ± 0.45*
Aragwadhadi kashaym (400mg/kg)	43.5 ± 2.07**	1.48 ± 0.12***	8.9 ± 0.26**

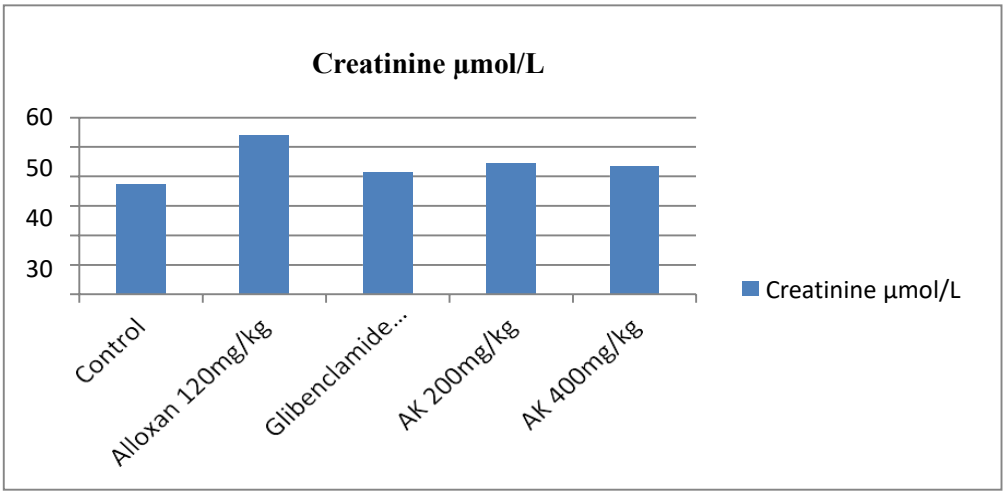


Fig 8: Effect of *Aragwadhadi kashayam* on Creatinine

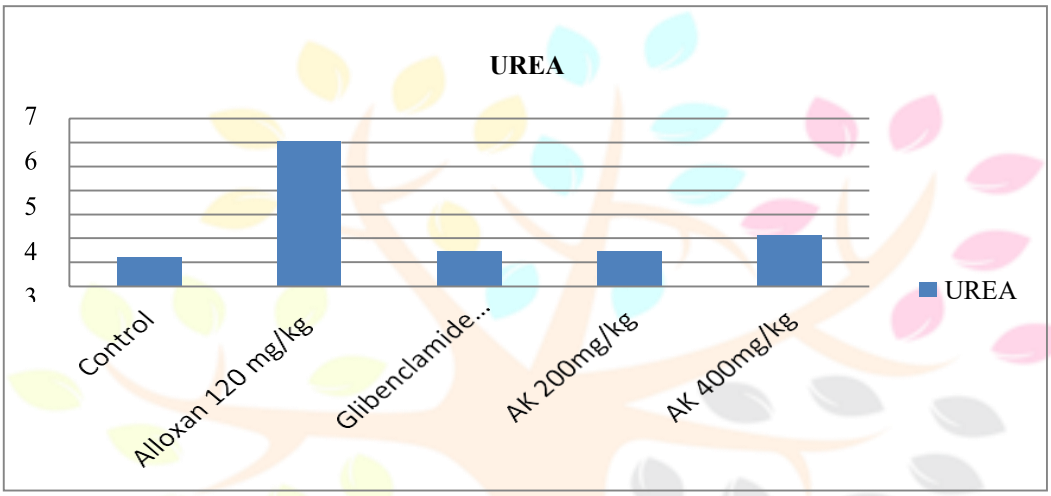


Fig 9: Effect of *Aragwadhadi kashayam* on Urea.

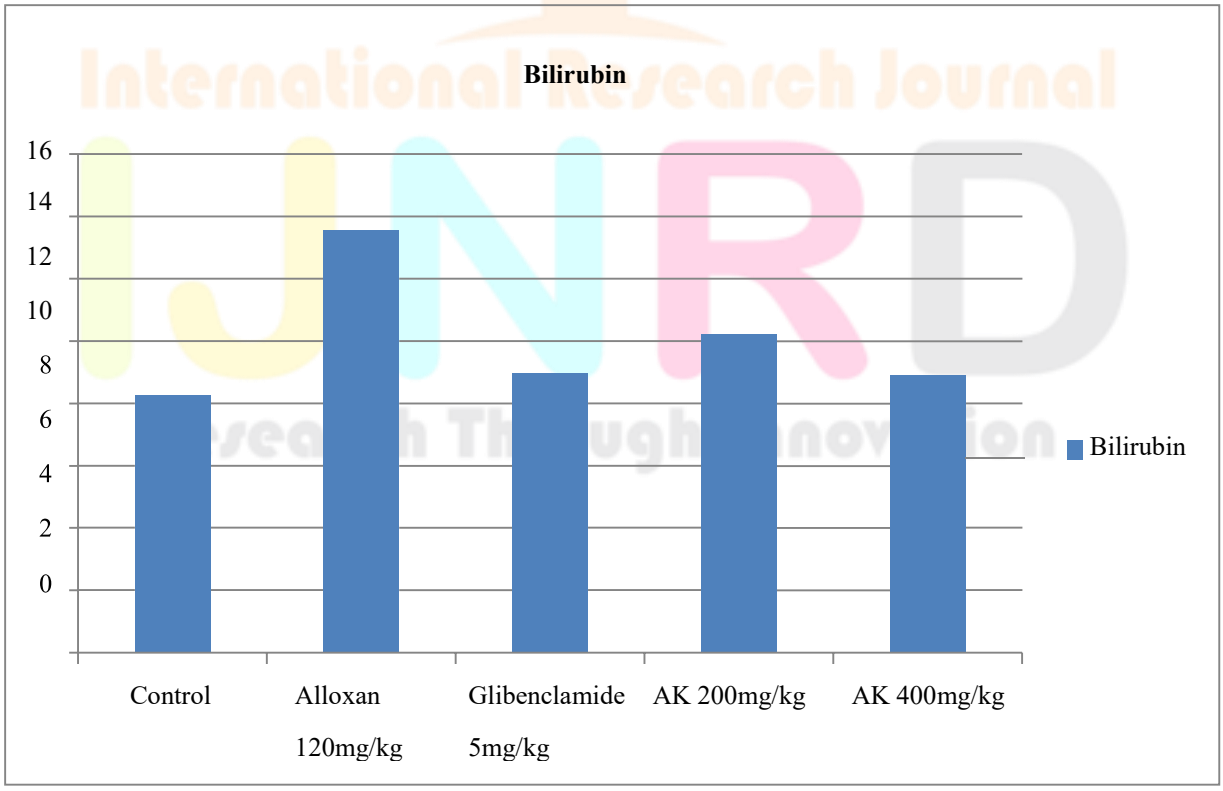
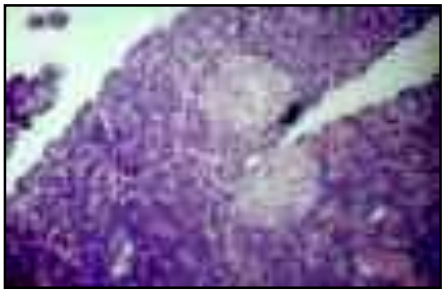


Fig10: Effect of *Aragwadhadi kashayam* on Bilirubin.

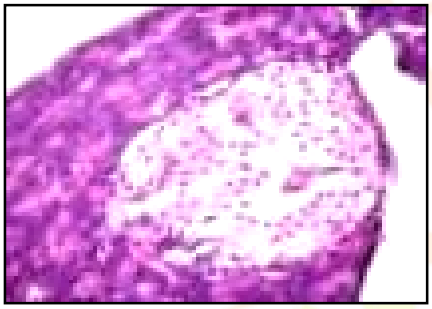
IV HISTOPATHOLOGICAL ASSESSMENT

Figure 11: T.S of Rat Pancreas



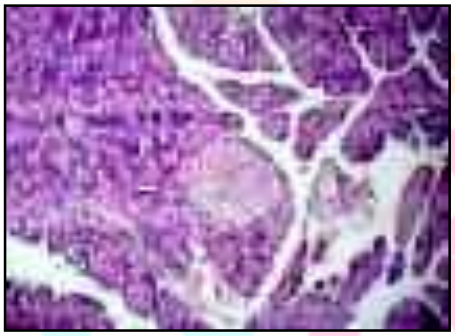
Control

Alloxan 120mg/kg



***Aragwadhadi kashayam* (200mg/kg)**

***Aragwadhadi kashaym* (400mg/kg)**



Glibenclamide 5mg/kg

V DISCUSSION

- Diabetes is a common chronic ailment for which the patient has to take insulin tomaintain the blood sugar level. It is very interesting to see how formulation tackles this problem. It corrects the function of pancreas, stimulating it to produce insulin in the natural way, which in turn maintains the blood sugar level. Formulation revitalizes and rejuvenates the organs, the dysfunction of which is causing the disease. This brings back normal functioning of the organs.It also maintains the healthy state of the body. Since no artificial chemicals are involved, it doesn't cause any side effects. [8]
- Qualitative phytochemical screening and ethnobotanical survey on the formulation revealed the presence of certain phyto constituents such as alkaloids, tannins, carbohydrates, glycosides, protein and amino acids, phytosterol, high amount of phenolic content, terpenoids and flavonoids.
- Diabetic mellitus (DM) is an endocrine disorder in which glucose metabolism is impairedbecause of total loss of insulin after

destruction of pancreatic beta cell or because of inadequate release of insulin from the pancreatic beta cell or insensitivity of target tissue to insulin. The fundamental mechanism underlying hyperglycemia involved over production (excessive hepatic glyconeogenesis and gluconeogenesis) and decrease utilization of glucose by the tissue. In the present study it was observed that whether the formulation have any effect on lipid profile or not and in addition to its antihyperglycemic action in Alloxan induced diabetic rats.[9]

- Phenolic compounds or phenolic phytohemicals are secondary metabolites of plant originand are important parts of the diet providing potential antioxidant benefits for managing oxidation stress-related chronic diseases such as diabetes.
- Dose dependant effects and Glibenclamide showed rapid normalization of blood glucose due to its insulin releasing effects.
- In our present study there was a significant weight gain in formulation treated diabetic rats compared with normal control rats and this observation shows anabolic effects of formulation on body weight on the diabetic rats. Hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of macrovascular complications. Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The hyperglycemia in diabetic might inhibit tissue repair in macrovacular beds. The formulation treatment increased the activity of enzymes and may thereby help to control free radicals, as formlation has been reported to be rich in total phenolic content, flavonoids and triterpenoids.
- Alloxan acts as diabetogenic by the destruction of beta cells of the islets of langerhans and causes massive reduction in insulin release, thereby inducing hyperglycaemia. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases etc. Intra- peritoneal (i.p) treatment of rats with alloxan monohydrate (120 mg/kg) significantly ($P<0.01$). *Aragwadhadi kashayam* and glibenclamide were found to reduce the elevated glucose level significantly in alloxan induced diabetes animals during 21 days treatment. The *Aragwadhadi kashayam* treatment has also increased the insulin level. So, one possible antidiabetic mechanismof *Aragwadhadi kashayam* may be stimulation of insulin secretion. A significant weight loss was observed in the diabetic group which was improved significantly by the *Aragwadhadi kashayam* treated groups as urinary glucose and protein release was restored after treatment.
- The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. Repeated administration of the *Aragwadhadi kashayam* for 21 days significantly ($P<0.01$) decreased hypertriglyceridemia and hypercholesterolemia, the observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis. HDL cholesterol level was significantly improved by the extract. Liver enzymes e.g. AST, ALT and ALP level were increased in diabetic rats which is responsible for the liver damage. The elevatedserum level of these enzymes was significantly reduced by *Aragwadhadi kashayam* treatment. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated enzymes. The restoration of transaminases to their normal levels also treatment also indicates revival of insulin secretion.
- The result of the present study showed that *Aragwadhadi kashayam* brings back the bloodglucose and body weight to normal in diabetes-induced rats. It also improved kidney, liver function and hyperlipidaemia due to diabetes. Antidiabetic action of the plant in diabetic ratsmay be possible through the insulinomimetic action or by other mechanism such as stimulationof glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscle.

VI CONCLUSION

- ✓ The presented study is an attempt to investigate the effect of *Aragwadhadi kashayam* on Alloxan induced diabetes in wistar albino rats.
- ✓ The Preliminary Phytochemical study was done which showed the presence of tannins, carbohydrate, flavonoids and reducing sugar which is responsible for the anti- diabetic activity.
- ✓ The animals were given Alloxan at a dose of 120mg/kg intraperitonially and the diabetic animals were treated with drug *Aragwadhadi kashayam* at a dose of 200mg/kg and 400mg/kg for21 days. The serum glucose, body weight, levels of Liver function tests, Haemoglobin content, Post Prandial glucose levels, AST, ALP, ALT, Urea, Creatinine, Total Bilirubin were significantly improved when compared with positive control group.
- ✓ All these observation imply that the *Aragwadhadi kashayam* could be regarded as an favorable Anti-diabetic agent. The

present investigation suggests that the *Aragwadhadi kashayam* has potential as an anti-diabetic agent against the Alloxan induced diabetes.

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