Section A-Research paper
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Triphala and Kaishore Guggul: A comprehensive choice to alleviate the diabetic and related disorders in obese condition.

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Abstract

Guggulu obtained from the stem and branches of Commiphora wightii belonging to the family Burseraceae. The different constituents are found in the plant such as volatile oils, gum E and Z Guggulsterones, Guggul sterols, Diterpenoids, Terpene, Cambrene, Myrcene, Dimyrcene and polymyrcene. The present study aimed to formulate and evaluated the guggulu formulations for the anti-diabetic activity and related disorders.

The animals were treated with high fat diet for 7 days. Type 1 diabetes was induced by injecting streptozotocin (STZ) of 65 mg/kg (in 10 mM ice-cold citrate buffer, pH 4.5) subcutaneously after subcutaneous injection of nicotinamide (NA, 230 mg/kg) (pH 4.5). A set of biochemical parameters were studied: glucose, cholesterol, triglycerides, HDL, LDL, VLDL, were evaluated. Serum marker of liver function such as such as levels of serum creatinine, urea, BUN, alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as

well as serum total proteins (TP) and albumin (ALB) were estimated, and the effect of formulations on insulin level.

It was concluded that Kaishore guggul kwatha, Kaishore guggul tablet were found to be effective compared with other formulations and standard.

Introduction

Guggulu consists of oleo-gum resin obtained as an exudates from the tapping of stem and branches of Commiphora wightii (Arnott) Bhandari [syn. Commiphora mukul Engl; Balsamodendron

mukul [Family, Burseraceae]. The plant is commonly known as guggul tree and is found in arid areas of India, Bangladesh, and Pakistan. Guggul contain volatile oils, gum E and Z Guggulsterones, Guggul sterols, Diterpenoids, Terpene, Cambrene, Myrcene, Dimyrcene and polymyrcene. Guggul contains a mixture of plant compounds, including steroids, essential oils, lignans, flavonoids, carbohydrates, and amino acids — all of which may be responsible for its various health effects [1-4]. Guggul possesses anti-inflammatory and antioxidant properties, it has been used in ancient medicine to protect against a variety of diseases. Guggulu has a long history of use in Ayurveda. The Atharvaveda is the earliest reference containing its medicinal and therapeutic properties [5].

According to Ayurveda, Guggul chiefly exists in nature in five different forms, which are: Krishnan (black); Peet Varn (Yellow); Rakt (blood red); Neel (blue); Kapish (light brown). Guggulu has been used to treat obesity, osteoarthritis, rheumatoid arthritis, gout, facial paralysis, sciatica, constipation, haemorrhoids, liver disorders, inflammation, cyst, cervical lymphadenitis, coronary thrombosis, anaemia, diabetes, urinary calculus, increased frequency and turbidity of urine, and skin diseases [6, 7]. It has a wide range of usefulness in indigenous medicine. It is astringent and antiseptic and acts as a bitter, stomachic, and carminative when taken internally. Like all oleo resins, it causes increase in number of leucocytes and stimulates phagocytosis. It acts as a diaphoretic, expectorant, diuretic, uterine stimulant, and emmenagogue. The resin is used in the form of lotion for indolent ulcers and as a gargle in caries, spongy gums, pyorrhea, chronic tonsillitis, and ulcerated throat. Inhalation of the fumes from burnt guggulu is recommended in hay fever, acute and chronic nasal catarrh, chronic laryngitis, chronic bronchitis, and phthisis. It is an ingredient of ointment for ulcers [8].

Triphala is a mixture of the dried fruits of plants including amla, bibhitaki, haritaki. It has been used in traditional ayurvedic medicine as a multi- purpose treatment. Triphala is used to treat common ailments such as inflammatory disorders, cancer, dental disease and cavities, constipation [9]. Triphala guggul kwatha is a traditional polyherbal formulation as per the Ayurvedic system of medicine. It is composed of resin of guggul (Commiphora wightii), long pepper (Piper longum) and Triphala (fruits of Phyllanthus emblica L., Terminalia chebula Retz, and Terminalia bellirica) [10]. Triphala Guggulu provides effective aid in the treatment of constipation. It works by stimulating the digestive fire which in turn relieves constipation. This further prevents piles and gives relief from the pain, swelling, and other discomfort associated with them. It also helps reduce the symptoms of anal fistula (fistula-in-ano). Triphala Guggulu can also help in weight management due to its laxative properties [11]. Kaishore guggulu mixture contains Gokshuru, Amruta, Guduchi, Tryushana, Vidanga, Danti, Trivrit, Ghrita and Trikatu [12]. Gokshuru is an herb that promotes natural urine flow. It also soothes membranes around the urinary tract. Trikatu is also added to support a healthy blood flow. It also improves digestive functions in the body. Kaishore Guggulu Tablets are used to reduce the total amount of uric acid in the body. This reduces the risk of inflammation and constipation among other bowl-related threats. It also makes it easier for a patient to expel urine. Administered in malignant ulcers, leprosy, boils, fistula, scitica, and otorrhoea. Kaishore guggul purifies blood. It is made from herbal extracts with natural anti-inflammatory properties. It is effective in soothing wounds and ulcers. It also boosts your digestive system [13-15].

Guggulu is a very potent medicines as it possess paranormal qualities, is particularly useful in treating a wide range of diseases. Therefore the present work was aimed to formulate the different formulations of guggulu and evaluated for their potential against diabetic and related disorders.

Material and Method

Material

The authentic raw material *Commiphora wightii* (guggul), *Terminalia chebula* (haritaki), *Terminalia bellirica* Roxb. (bibhitaki), *Emblica officinalis* Gareth (amalaki), *Piper longum* (pippali), *Tinospora cordifolia* (guduchi), *Zingiber officinale* Roscoe (sunthi), *Piper nigrum* (marica), *Embelia ribes* (vidanga), *Operculina turpethum* (trivrutmul), *Baliospermum montanum*

(dantimool), *Achyranthes aspera* (aspera) were procured as gift samples from Amsar Pvt. Ltd. Colvale Goa with COA of each raw sample.

The other ingredients ghee (ghrut), talc, pulverized sugar were procured from local market.

Chemicals including Hydrochloric acid, chloroform, ethanol, sabourandschloramphenecol agar, methanol, ethyl acetate, potassium permanganate, indigo sulphonic acid were purchased from S. G. Fine.

Animal

The Institutional Animal Ethical Committee (IAEC) of Rajaram and Tarabai Bandekar college of Pharmacy, Ponda, India, authorized the experimental protocol with approval no. PES'RTBCOP/IAEC:clear2021R-91 and PES'RTBCOP/IAEC:clear2022R-97 for evaluation of hyperlipidemia and hepatotoxicity and nephropathic activity respectively using Wistar rats (150-170 gm) of either sex were procured for the experimental work from Global Bioresearch, Shirol.

Method

Formulation of kwatha and related formulations

Formula and method for preparation of Triphala kalpa

Table 1: Formula for triphala kwatha

Sr. No.	Name of Ingredients	Quantity taken
1.	Haritaki	33 gm
2.	Bibhitaki	33 gm
3.	Amalaki	33 gm
4.	Water	800 ml

Procedure:

All the ingredients stated in Table 1 were powdered and boiled in demineralized water to acquire aqueous extract. The extract was concentrated to its $1/4^{th}$ volume of its initial quantity. The obtained thick parte (kwath) was filtered through filter cloth.

Formula and method for preparation of Triphala guggul

Table 2: Formula for triphalaguggulkalpa gutikas

Sr. No.	Name of Ingredients	Quantity taken
1.	Haritaki	48gm
2.	Bibhitaki	48gm
3.	Amalaki	48gm
4.	Pippali	48gm
5.	Guggulu- suddha	240gm

Weigh all the finely powdered drugs including haritaki, bibhitaki, amalaki and pippali and added to guggulu- suddha and pounded well.

Formula and method for Preparation of Kaishore Guggul Kwatha

Table 3: Formula for Kaishore guggul tablets

Sr. No.	Name of Ingredients	Quantity taken
1.	Guggulu- suddha	768gm
2.	Haritaki	256gm
3.	Bibhitaki	256gm
4.	Amalaki	256gm
5.	Guduchi	1.536kg
6.	Water for decoction	12.288 lit
	Reduced to	6.144 lit
7.	Haritaki	8gm
8.	Bibhitaki	8gm
9.	Amalaki	8gm
10.	Sunthi	24gm
11.	Marica	24gm
12.	Pippali	24gm

13.	Vidanga	24gm
14.	Trivrutmul	12gm
15.	Dandimul	12gm
16.	Guduchi	48gm
17.	Grut (qs)	384gm

The kasaya of drugs harikati, bibhitaki, amalaki, guduchi (drugs 2- 5) was prepared. Guggulu was added to the filtered kasaya and boiled in an iron vessel until the mixture becomes concentrated. The remaining finely powdered drugs were added and stirred well. At the end of process ghrta was added and mixed well. Press the mass under tablet compression machine to produced desired size of tablets.

Formula and Method for Preparation of Kaishore Guggul Pills

Table 4: Formula for Kaishore guggul pills

Sr. No.	Name of Ingredients	Quantity taken
1.	Guggulu- suddha	768gm
2.	Haritaki	256gm
3.	Bibhitaki	256gm
4.	Amalaki	256gm
5.	Guduchi	1.536kg
6.	Water for decoction	12.288 lit
	Reduced to	6.144 lit
7.	Haritaki	8gm
8.	Bibhitaki	8gm
9.	Amalaki	8gm
10.	Sunthi	24gm
11.	Marica	24gm
12.	Pippali	24gm
13.	Vidanga	24gm

14.	Trivrutmul	12gm
15.	Dandimul	12gm
16.	Guduchi	48gm
17.	Grut (qs)	384gm

Followed the procedure given for preparation of Kaishore Guggul Kwatha. At the end of process ghrta was added and mixed well. Then pills were rolled.

Evaluation of formulation

The kwatha formulations were evaluated for its organoleptic characteristics including color, odor, taste, percent yield, and loss on drying. The pills/ tablets were evaluated for hardness, disintegration time, and weight variation test.

Evaluation of formulations for Anti-diabetic and related disorders (hyperlipidemia, hepatotoxicity and nephropathic) activity

Eight week old male Wistar rats (150- 170 gm) were procured for the experimental work from Global Bioresearch, Shirol. The animals were maintained in proper cages at 22°C ± 1°C, 60% ± 10% RH, with a change in 12 hrs of day/night and fed ad libitum with a laboratory chow for a week before the experiments started. The animals were treated with high fat diet for 7 days. Type 1 diabetes was induced by injecting streptozotocin (STZ) of 65 mg/kg (in 10 mM ice-cold citrate buffer, pH 4.5) subcutaneously after subcutaneous injection of nicotinamide (NA, 230 mg/kg) (pH 4.5). After administration of STZ animals were fed with high fat diet containing 40% energy from fat as well as consequently another dose of NA and STZ was administered after 24 hrs. At the same time rats in control group were treated citrate buffer injection alone with same route of administration. Seven days later, the rats were evaluated for the fasting blood glucose levels and the rats that showed blood glucose level above 220 mg/ dL considered as DM. Then the rats were randomly segregated in different eight groups.

Group I: Normal group

Group II: HFD group treated with STZ (35mg/kg)

Group III: Guggul (100mg/kg)

Group IV: Kaishore guggul Kwatha (100mg/kg)

Group V: Kaishore guggul tablet (100mg/kg)

Group VI: Triphala kwatha(100mg/kg)

Group VII: Triphala guggul kalpa Tablets (100mg/kg)

Group VIII: Glibenclamide (5 mg/kg)

The study was carried out for next 28 days. Each day, the groups were fed with respective test sample orally. At the end of the experimental period, the blood samples were collected on ethylenediamine tetra-acetic acid containing tubes and serum was evaluated immediately for following parameters.

Biochemical Estimations

At the end of the experimental day, the blood samples were collected on ethylene diamine tetra-acetic acid containing tubes and serum was separated immediately. Fasting and postprandial glucose levels (FBS and PPBS), serum lipid profiles were measured. The insulin level was measured using enzyme-linked immunosorbent assay kit.

- **Determination of Kidney Weight**

At sacrifice, the absolute kidney weight was determined using a top loader sensitive balance (Mettler Toledo Germany). The relative weight of the kidney (%) was calculated from the body weight at sacrifice and the absolute kidney weights as previously described.

$$\text{Relative weight of the kidney} = \frac{\text{Absolute weight}}{\text{body weight at sacrifice}} \times 100$$

- **Serum lipid profiles**

Blood samples were obtained from the rats by cardiac puncture at sacrifice and were kept for 30 min at room temperature. Serum was separated from the blood samples by centrifugation at 5000 rpm for 10 min at room temperature. After 30 days of STZ administration, the level of serum total cholesterol, triglycerides, HDL, LDL, VLDL, were evaluated. Serum marker of liver function such as such as levels of serum creatinine, urea, BUN, alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as well as serum total proteins (TP) and albumin (ALB) were estimated spectrophotometrically, using enzymatic colorimetric assay kits (Randox, Northern Ireland) following standard methods.

- **Serum Insulin level was measured using enzyme-linked immuno-sorbent assay kit.**

ELISA plates (96 wells) were coated with specific mouse insulin (100 µl/well) and incubated overnight at 4°C. The assay diluents (200 µl/well) were utilized to inhibit the plate's non-specific protein-binding sites. Immediately, 100 µl of culture supernatant or the appropriate standard was

added to the appropriately coated wells and incubated at room temperature for 2 hours. Following incubation, the plates were thoroughly rinsed 5 times with wash buffer [phosphate-buffered saline (PBS) with 0.05 percent Tween-20]. Each well received 100 µl of detecting solution (detection antibody and streptavidin horse-radish peroxidase).

The plates were carefully sealed with a plate sealer and incubated for 1 hour at room temperature before being completely washed 5 times with wash buffer. A 100 µl tetramethylbenzidine (TMB) substrate solution was added to each well, and the plate was incubated for 30 minutes in the dark at room temperature (without the plate sealer). Finally, each well received 50 µl of stop solution (2N H_2SO_4). ELISA data were obtained using an ELISA reader at 450 nm (Bio-Rad Laboratories, CA, USA). The concentration of each cytokine was measured in three wells, and the results were derived from the standard curve and expressed as pg/ml.

- **Histopathology of pancreas, Liver and kidney**

The histopathology study was performed on harvested Pancreas, Liver and kidney to evaluate the effect of formulations. For histopathological examination pancreas, kidney and liver from each treatment group were fixed in formalin solution (10% v/v) for 24 h, bisected longitudinally and embedded in paraffin. Sections of 4–6 µm thickness were cut stained by aqueous hematoxylin and alcoholic eosin and were examined by bright field microscopy (Olympus, India).

Result and Discussion

Material

The authentic raw material *Commiphorawightii*(guggul), *Terminaliachebula* (haritaki), *Terminaliabellirica*Roxb. (bibhitaki), *Emblicaofficinalis* Gareth (amalaki), *Piper longum* (pippali), *Tinospora cordifolia* (guduchi), *Zingiberofficinale*Roscoe (sunthi), *Piper nigrum* (marica), *Embeliaribes* (vidanga), *Operculinaturpethum* (trivrutmul), *Baliospermummontanum* (dantimool), *Achyranthesaspera* (aspera)were gifted from Amsar Pvt. Ltd. Colvale Goa.

Method

Evaluation of Formulation

The prepared formulations including Triphala kwatha, Triphala guggul kalpa, kaishore guggul kwatha, kaishore guggul tablet were evaluated for different parameters given in table 5, 6, 7, 8.

Table 5: Evaluation of Triphala kwatha

Sr. No.	Parameters	Obtained Values
1.	Organoleptic Characters	
	Colour	Blackish brown
	Odour	Characteristic odour
	Taste	Kashaya, Tikta, Amla
2.	Percentage yield	40%
3.	Loss on dying	2.25 w/w
4.	pH	4.20@23^{0}C
5.	Total ash	4.35%
6.	Acid insoluble ash	0.22%
7.	Water soluble extractives	85.26%
8.	Alcohol soluble extractives	54.28%
9.	Moisture Content (Loss on Drying at 105°C)	15.85%

Table 6: Evaluation of Triphala guggul kalpagutikas/ Tablet/ Pills

Sr. No.	Parameters	Values
1.	Percentage yield of gallic acid	0.5%
2.	Hardness (Kg/Cm^2)	3-4
3.	Disintegration time	60
4.	Weight variation test	Pass

Table 7: Evaluation of Kaishore guggul Kwatha

Sr. No.	Parameters	Values
1.	Organoleptic Characters	
	Colour	Blackish brown
	Odour	Characteristic odour
	Taste	Kashaya, Amla
2.	Percentage yield	40%
3.	Loss on Drying	2.20 w/w
4.	pH	4.18@23^{0}C
5.	Total ash	4.15%
6.	Acid insoluble ash	0.25%
7.	Water soluble extractives	84.60%
8.	Alcohol soluble extractives	52.28%
9.	Moisture Content (Loss on Drying at 105°C)	14.56%

Table 8: Evaluation of kaishore guggul tablets/ pills

Sr. No.	Parameters	Values
1.	Percentage yield	40%
2.	Hardness (Kg/Cm^2)	2-3
3.	Disintegration time	60
4.	Weight variation test	Pass

Animal Study

Anti-diabetic and related disorders (hyperlipidemia, hepatotoxicity and nephropathic) activity of different guggul formulations in high fat diet with streptozotocin induced diabetic model

Insulin resistance is seen in the majority of persons who suffer from metabolic syndrome. Insulin is produced by the body in order to transport glucose (sugar) into the cells, where it can be used as a source of energy. It is more difficult for cells in the body to respond to insulin when there is obesity present, which is a common finding in persons who have metabolic syndrome. The development of type 2 diabetes can occur when the body is unable to produce enough insulin to overcome insulin resistance, which results in an increase in blood sugar levels. The anti-diabetic action was studied by inducing diabetes with streptozotocin. The rats were evaluated for change in kidney weight and fasting blood glucose level.

Evaluation of Anti-diabetic Effect

From the obtained data it was concluded that the formulation containing kaishore guggul i.e. kaishore guggul kwatha and kaishore guggul tablet were found to be effective in reducing blood glucose level as well as kidney weight compared with other dosage forms. However, the formulations also reduced the recurring chance of hepatotoxicity as well as nephropathy.

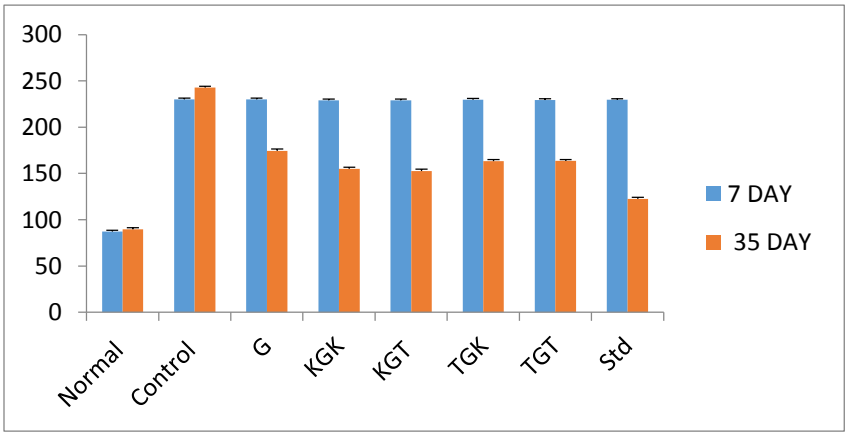

Figure 1: Change in Blood glucose level (G: Guggul; KGK: Kaishore guggul kwatha; KGT: Kaishore guggul tablet; TGK: Triphala guggul kwatha; TGT: Triphala guggul tablet)

Evaluation of Anti-hyperlipidemia Effect

The effect of formulations on the serum lipid profile was given in table 9. The formulations prepared with kaishore guggul kwatha (156.40±1.75) and kaishore guggul tablet (156.27±1.61) showed significant decreased in the serum total cholesterol level when compared with rats in standard group. The formulations prepared with kaishore guggul kwatha (54.52±1.55) and kaishore guggul tablet (54.68±1.49) showed significant decreased in the serum triglyceride level when compared with rats in standard group. The formulations prepared with kaishore guggul kwatha (54.52±1.55) and kaishore guggul tablet (54.68±1.49) showed significant increase in the serum HDL level when compared with rats in standard group. The formulations such as kaishore guggul kwatha and kaishore guggul tablet were found to be effective in reducing LDL level in serum. The formulations prepared with kaishore guggul kwatha (76.29±1.45) and kaishore guggul tablet (75.20±1.91) showed significant decreased in the serum VLDL level when compared with rats in standard group.

Table 9: Effect of formulations on Serum lipid profile

Groups	TC	TG	HDL	LDL	VLDL
	mg/dL				
Normal	113.66±1.05	23.1±0.45	46.51±1.18	52.99±0.82	15.63±0.16
Control (HFD with STZ	286.76±2.05	108.28±0.7	18.90±1.0	113.53±0.	57.56±1.3

35 mg/kg)		6	8	68	5
Guggul (100 mg/kg)	174.88±1.81	74.68±1.83	29.18±1.61	94.67±0.68	35.55±1.74
Kaishore guggul Kwatha (100mg/kg)	156.40±1.75	54.52±1.55	38.14±0.81	80.98±0.76	24.04±0.58
Kaishore guggul tablet (100mg/kg)	156.27±1.61	54.68±1.49	38.21±0.51	80.96±0.61	24.44±0.84
Triphala kwatha (100mg/kg)	166.14±1.48	56.48±1.51	32.78±1.68	76.29±1.45	33.39±1.26
Triphala guggul kalpa Tablets (100mg/kg)	166.19±1.67	56.67±1.61	34.31±2.05	75.20±1.91	34.28±0.95
Standard (Glibenclamide 5 mg/kg)	167.53±2.67	78.7±1.69	27.74±0.75	95.90±1.11	41.37±0.93

Effect of formulations on Kidney function

The effect of formulations on kidney function is given in table 10. The formulations prepared with kaishore guggul kwatha showed significant decreased in serum creatinine, urea, BUN level by 1.47±0.22, 33.33±1.03, 18.12±0.93 respectively whereas kaishore guggul tablet showed significant decreased in the serum creatinine, urea, BUN level by1.33±0.12, 35.60±1.66, 18.03±0.64 respectively when compared with rats in standard group.

Table 10: Effect of formulations on Kidney function

Groups	Creatinine	Urea	BUN
Normal	0.81±0.03	28.14±2.36	15.01±1.10
Control (HFD with STZ 35 mg/kg)	4.05±0.44	74.87±1.21	34.96±2.27
Guggul (100 mg/kg)	2.30±0.28	44.47±1.58	28.43±0.96
Kaishore guggul Kwatha (100mg/kg)	1.47±0.22	33.33±1.03	18.12±0.93
Kaishore guggul tablet (100mg/kg)	1.33±0.12	35.60±1.66	18.03±0.64
Triphala kwatha (100mg/kg)	1.63±0.12	38.43±1.67	20.53±0.48
Triphala guggul kalpa Tablets	1.67±0.16	36.72±2.94	20.10±0.74

(100mg/kg)			
Standard (Glibenclamide 5 mg/kg)	3.42±0.15	54.26±1.10	33.70±0.53

Effect of formulations on liver functioning test

Table 11 summarized the effect of prepared formulations on liver. The Kaishore guggul Kwatha significantly reduce ALT, AST, ALP, ALB, and TP by 60.25±1.41, 56.81±1.09, 59.58±2.68, 0.58±0.05 and 0.59±0.05 respectively whereas Kaishore guggul tablet reduce ALT, AST, ALP, ALB, and TP by 54.85±1.05, 52.74±1.08, 59.26±0.89, 0.59±0.05, and 0.58±0.02 respectively. The study concluded that kaishore guggul tablet were found to be effective in reducing ALT, AST, ALP, ALB and TP level than kaishore guggul kwatha.

Table 11: Effect of formulations on liver functioning test

Groups	ALT	AST	ALP	ALB	TP
Normal	44.81±1.12	46.22±1.06	51.22±0.74	0.35±0.01	0.86±1.29
Control (HFD with STZ 35 mg/kg)	118.61±1.62	172.20±1.22	103.40±1.22	1.63±0.09	1.66±0.10
Guggul (100 mg/kg)	65.24±1.56	71.29±1.12	71.74±1.29	0.84±0.36	0.76±0.04
Kaishore guggul Kwatha (100mg/kg)	60.25±1.41	56.81±1.09	59.58±2.68	0.58±0.05	0.59±0.05
Kaishore guggul tablet (100mg/kg)	54.85±1.05	52.74±1.08	59.26±0.89	0.59±0.05	0.58±0.02
Triphala kwatha (100mg/kg)	62.88±0.94	59.02±0.89	66.05±0.90	0.67±0.04	0.64±0.03
Triphala guggul kalpa Tablets (100mg/kg)	57.05±1.06	67.45±1.03	64.27±1.49	0.66±0.05	0.65±0.02
Standard (Glibenclamide 5 mg/kg)	55.60±1.05	65.37±0.66	65.73±1.36	0.65±0.02	0.60±0.02

Serum Insulin level was measured using enzyme-linked immuno-sorbent assay kit

ELISA was used to assess the influence of formulations on the production of insulin. The mean post-treatment serum insulin level of the normal group (85.95 ±1.78 pMol/L), was significantly higher ($p < 0.001$) than the control group (29.99 ±1.26 pMol/L). The guggul formulations kaishore guggul kwatha, kaishore guggul tablet, Triphala guggul kwatha and Triphala guggul tablet at dose 100 mg/kg treated groups exhibited a dose-dependent improvement in serum insulin levels with the Triphala guggul tablet demonstrating the highest mean serum insulin levels (80.23±1.41pMol/L) and significantly higher than the model ($p < 0.001$) (Figure 2). Diabetic rats showed a significant decrease in plasma insulin compared with normal rats.

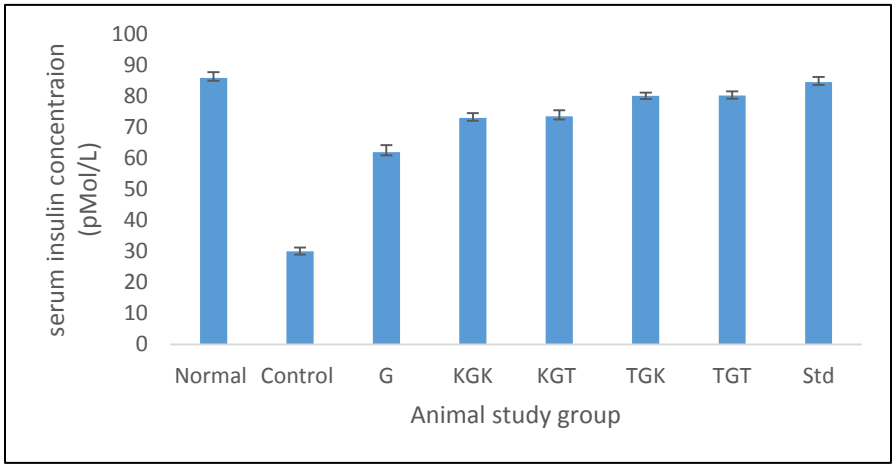

Figure 2: Effect of formulations on serum insulin concentration (G: Guggul; KGK: Kaishore guggul kwatha; KGT: Kaishore guggul tablet; TGK: Triphala guggul kwatha; TGT: Triphala guggul tablet)

Histopathology of pancreas, liver and kidney

Histopathology of Pancreas

Effect of formulations on the histological profile of the pancreas in untreated normal, HFD-STZ-induced diabetic wistar rats was evaluated. (See Figure A.) hematoxylin and eosin (H/E) stained slices of pancreas from a normal control rat showing normal islet of langerhans, indicated by orange arrows. (See Figure B.) Pancreatic slice of an HFD-STZ-induced diabetic rat

demonstrating no/destroyed islet of langerhans and beta cells, as shown by yellow arrows. (See Figure C.) Pancreatic slice of HFD-STZ-induced diabetic rats treated with guggul demonstrating very small islet of langerhans withfew beta cells and inflammation indicated by blue arrow. (See Figure D, E, F, G and H) Pancreatic slice of HFD-STZ-induced diabetic rats treated with Kaishore guggul kwatha, Kaishore guggul tablet, Triphala guggul kwatha, Triphala guggul tablet, Glibenclamide demonstrating small number of normal islet of langerhans with numerous beta cells with few spots of inflammation (green arrows).

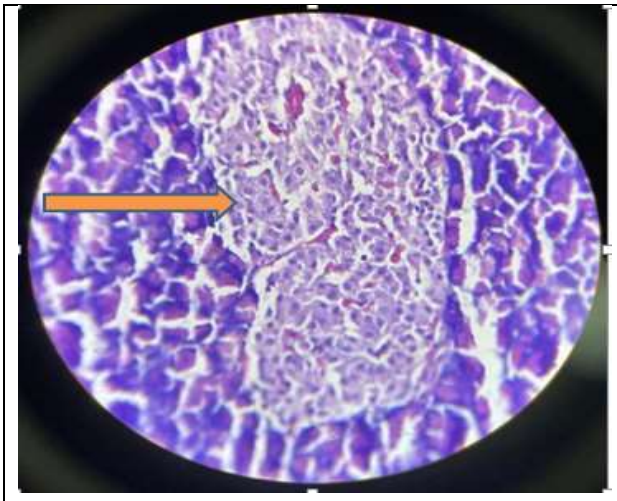

Figure A: Normal

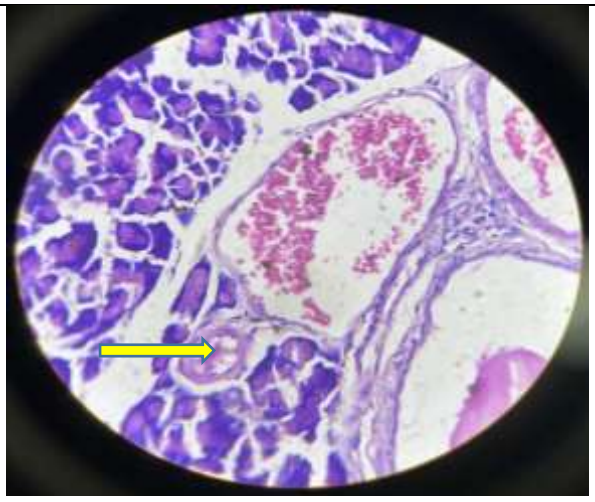

Figure B: Control (HFD-STZ)

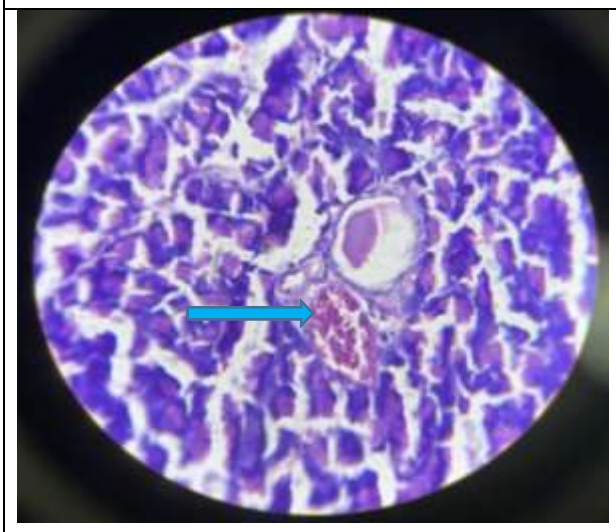

Figure C: Guggul

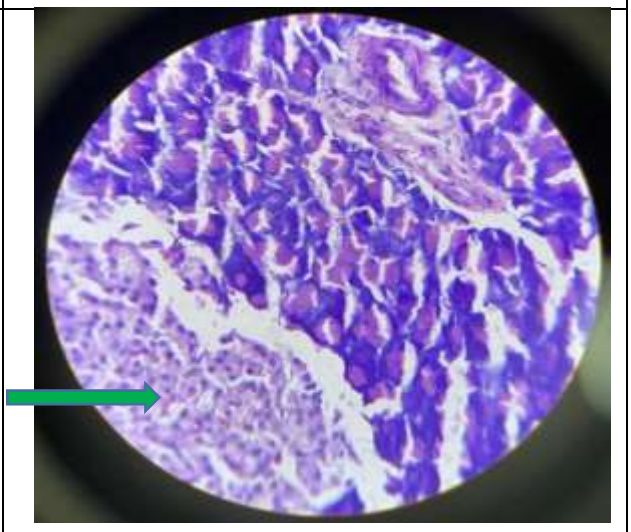

Figure D: Kaishore guggul kwatha

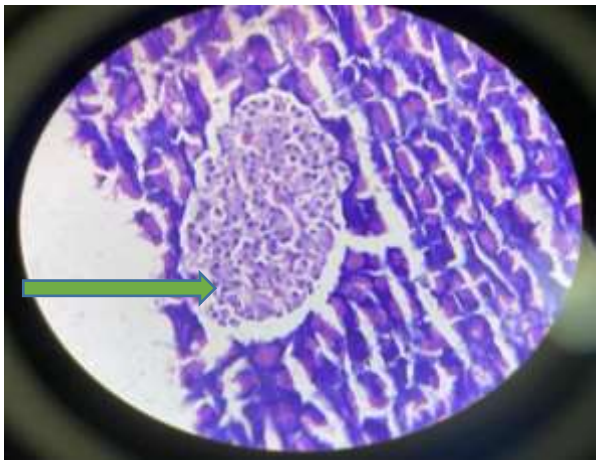

Figure E: Kaishore guggul tablet

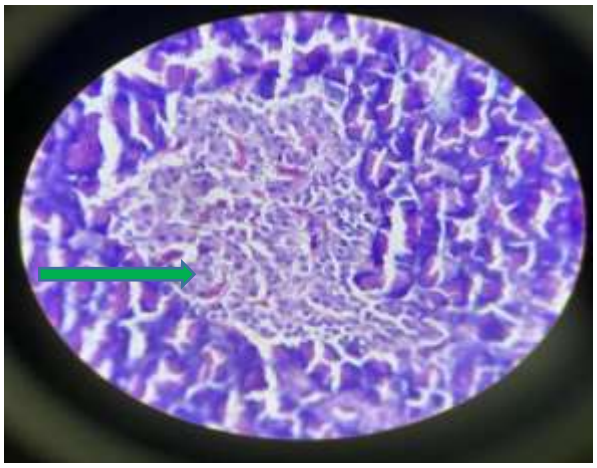

Figure F: Triphala guggul kwatha

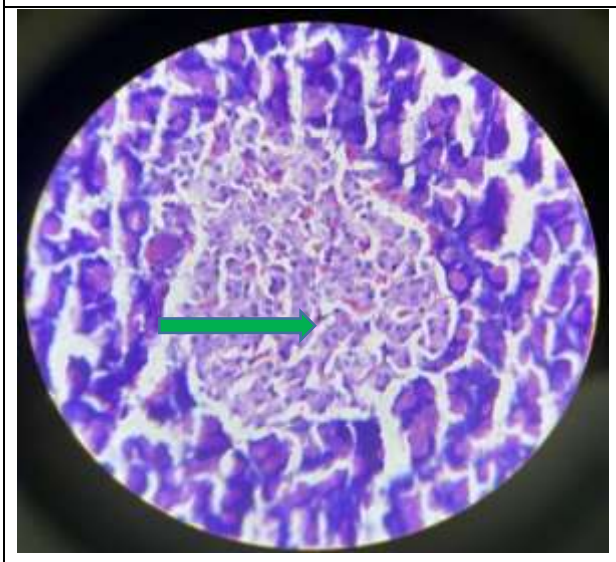

Figure G: Triphala guggul tablet

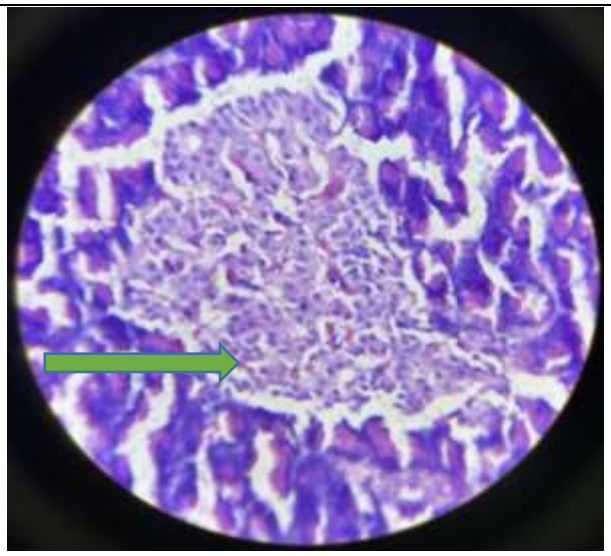

Figure H:Glibenclamide

Figure 4: Results of Histopathology of Pancreas

Note- Group A: Animals treated with Normal diet and no induction; Group B: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks); Group C: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Guggul; Group D: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul kwatha; Group E: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul tablet; Group F: Animals treated

with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Triphala guggul kwatha; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Triphala guggul tablet; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Glibenclamide

Orange arrow: normal islet of Langerhans

Yellow arrow: no/destroyed islet of langerhans and beta cells

Blue arrow: very small islet of langerhans withfew beta cells and inflammation

Green arrow: small number of normal islet of langerhans with numerous beta cells with few spots of inflammation

Histopathology of Liver

Histopathology of liver tissue of rats treated with high fat diet and HFD- streptozotocin induced diabetes untreated rats showed infiltered cells (Figure B, C, F, G and H) when compared to normal healthy control rats liver tissue which showed normal hepatocyte and normal sinusoids (Figure A). There was decreased in the number of inflammatory tissue which further results in infiltered cells observed (figure C, F G and H). While there was no infiltered cells were observed in the group of animals treated with kaishore guggul kwatha and kaishore guggul tablet (figure D, E). This concluded that the formulations kaishore guggul kwatha and kaishore guggul tablet were found be effective in treating liver related disorders.

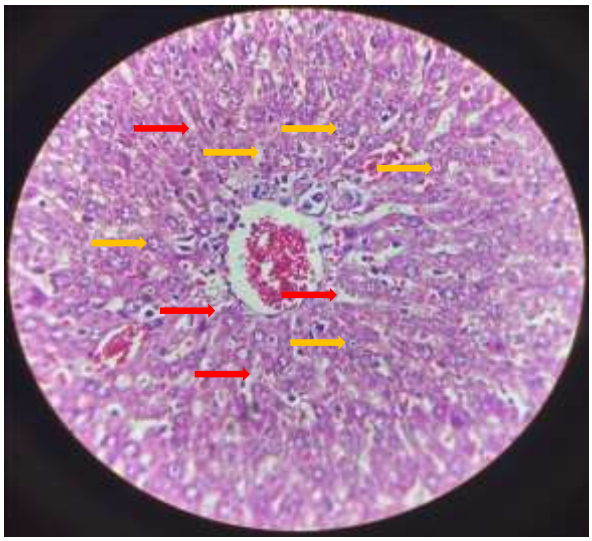

Figure A: Normal

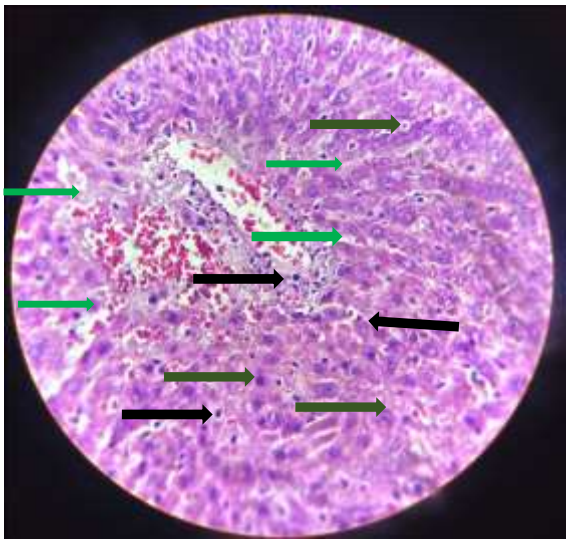

Figure B: Control (HFD-STZ)

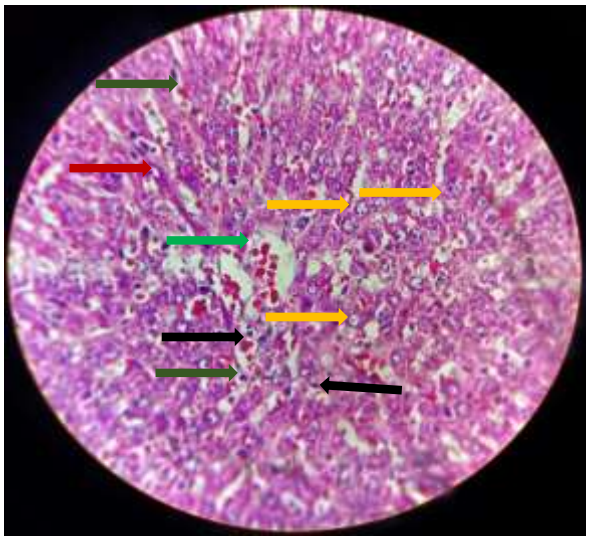

Figure C: Guggul

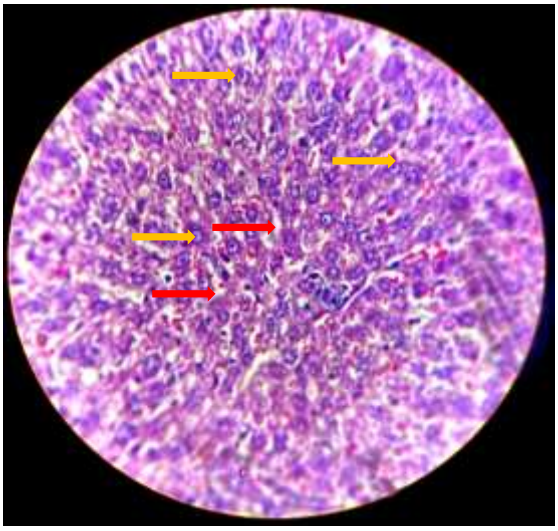

Figure D: Kaishore guggul kwatha

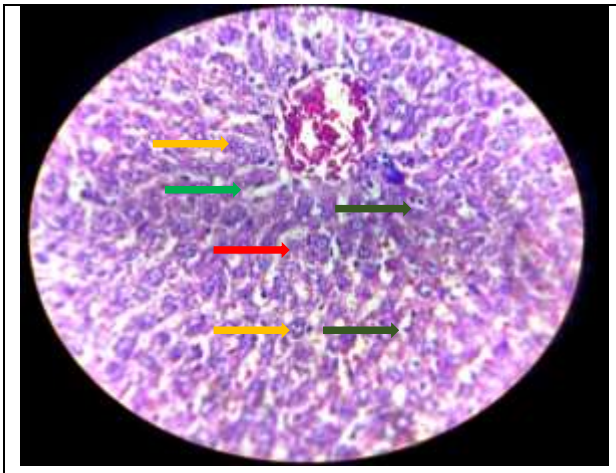

Figure E: Kaishore guggul tablet

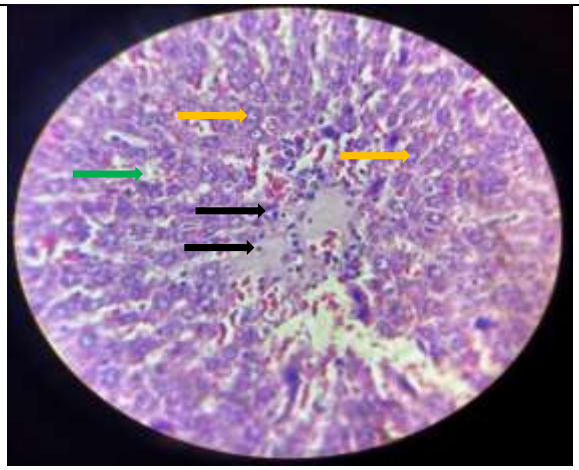

Figure F: Triphala guggul kwatha

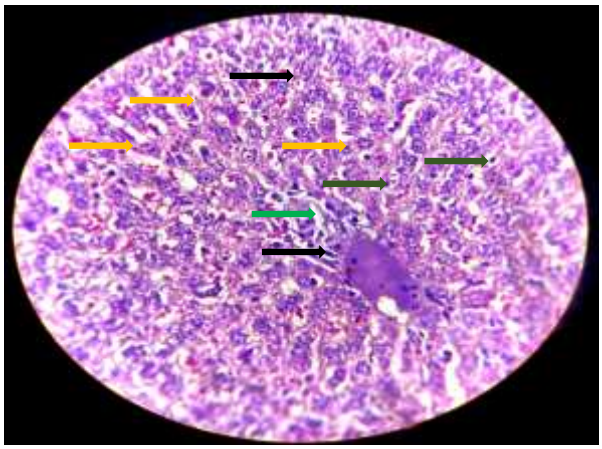

Figure G: Triphala guggul tablet

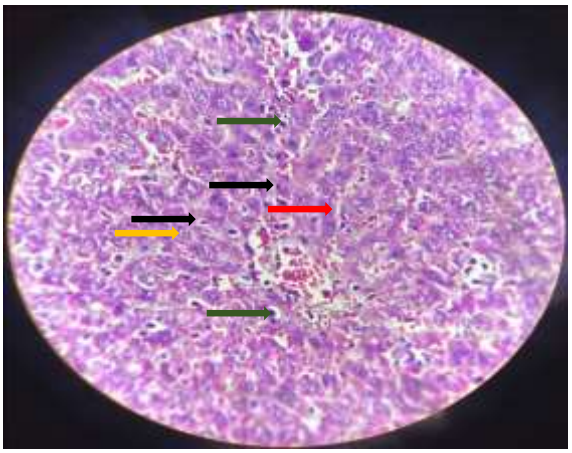

Figure H: Glibenclamide

Figure 3 A-H: Results of Histopathology of Liver

Note- Group A: Animals treated with Normal diet and no induction; Group B: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks); Group C: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Guggul; Group D: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul kwatha; Group E: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul tablet; Group F: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then

treated with Triphala guggul kwatha; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Triphala guggul tablet; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Glibenclamide

Yellow arrow: normal hepatocytes

Red arrow: Normal sinusoids

Brown arrow: degenerated liver

Green arrow: Dilated sinusoids

Black arrow: infiltration cells (sign of inflammation)

Histopathology of Kidney

Effect of formulations on the histological profile of the kidney in untreated normal, HFD- STZ-induced diabetic wistar rats was evaluated. (See Figure A.) Tissue of kidney showing normal glomeruli cells indicated by orange arrows. (See Figure B.) Kidney slice of an HFD-STZ-induced diabetic rat demonstrating no/destroyed glomeruli cells and heavy load of inflammatory cells in kidney, as shown by yellow arrows. (See Figure C, F and G) Kidney tissue of HFD-STZ-induced diabetic rats treated with guggul, Triphala guggul kwatha, triphala guggul tablet demonstrating no/destroyed glomeruli cells and heavy load of inflammatory cells in kidney (yellow arrow) and small glomeruli cells and infiltration of inflammatory cells in kidney (green arrow). (See Figure D, E and H) kidney tissue of HFD-STZ-induced diabetic rats treated with Kaishore guggul kwatha, Kaishore guggul tablet and Glibenclamide demonstrating normal glomeruli cells indicated with orange arrow. The results concluded that Kaishore guggul kwatha, Kaishore guggul tablet were found to be effective in treating kidney disorders.

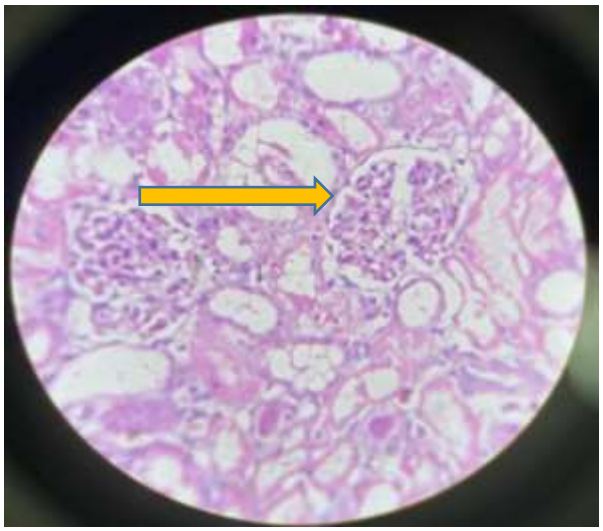

Figure A: Normal

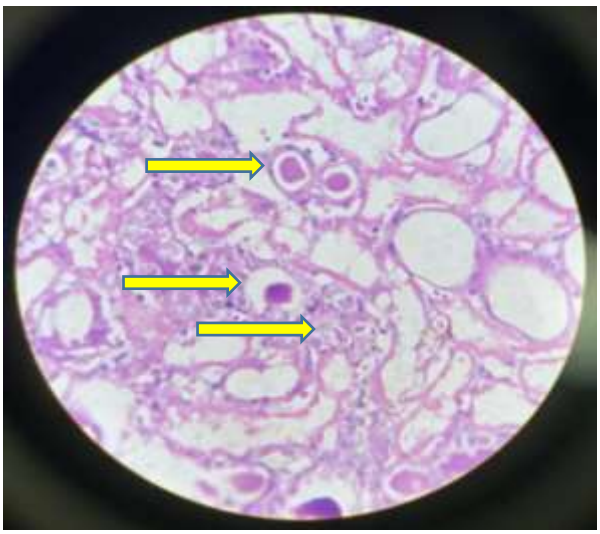

Figure B: Control (HFD-STZ)

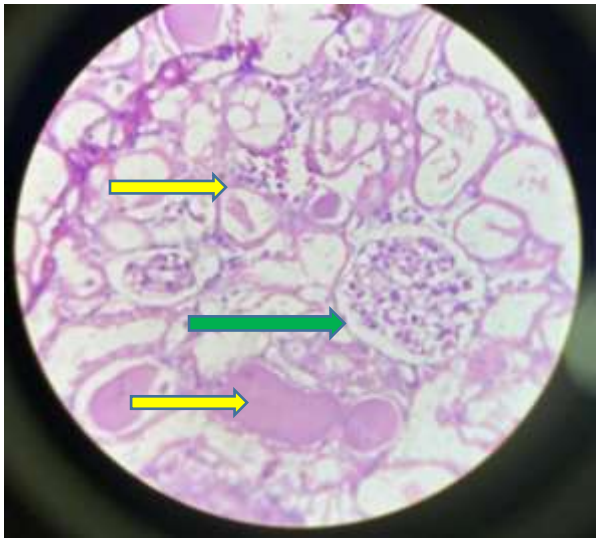

Figure C: Guggul

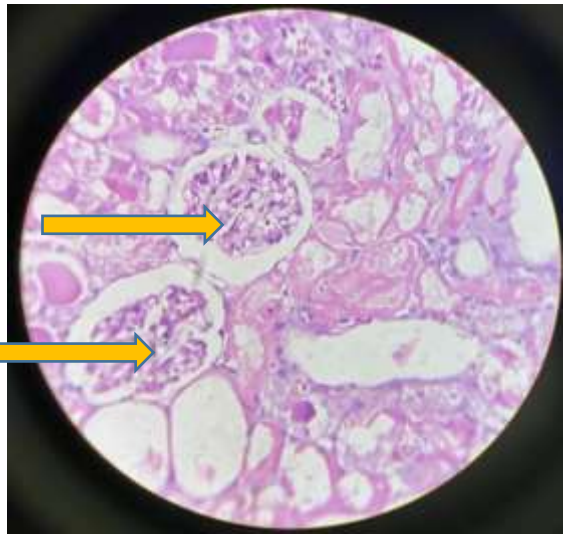

Figure D: Kaishore guggul kwatha

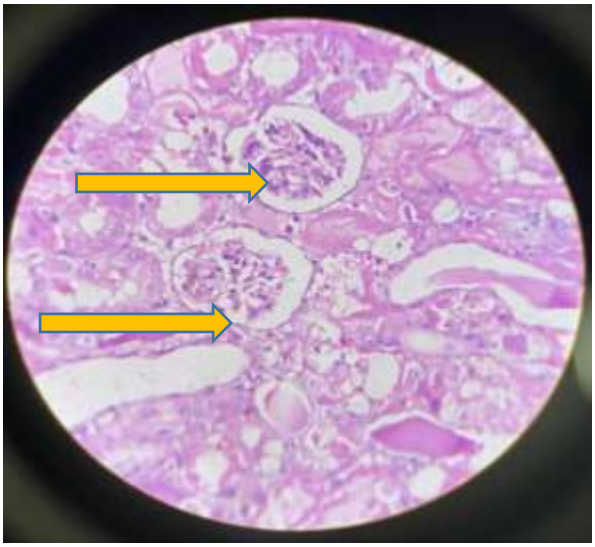

Figure E: Kaishore guggul tablet

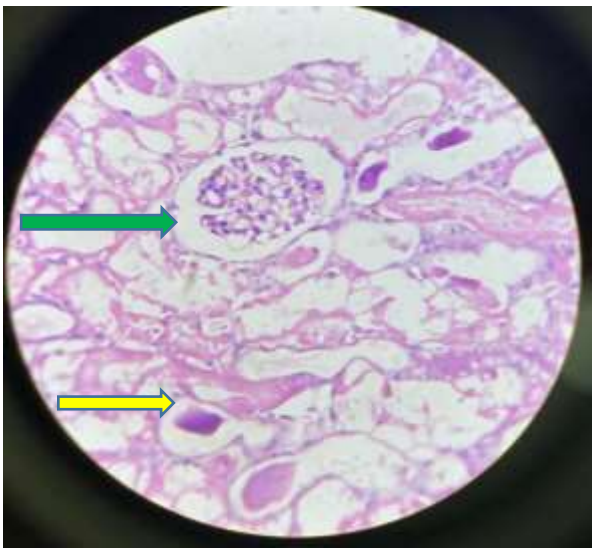

Figure F: Triphala guggul kwatha

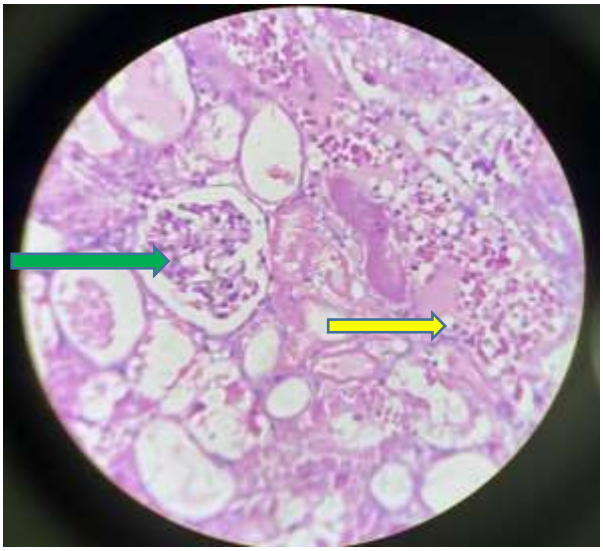

Figure G: Triphala guggul tablet

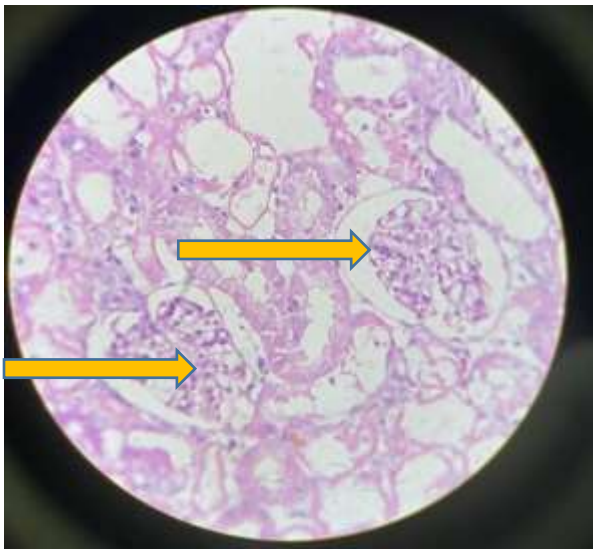

Figure H:Glibenclamide

Figure 5: Results of Histopathology of Kidney

Note- Group A: Animals treated with Normal diet and no induction; Group B: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks); Group C: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet

(4 weeks) then treated with Guggul; Group D: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul kwatha; Group E: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Kaishore guggul tablet; Group F: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Triphala guggul kwatha; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Triphala guggul tablet; Group G: Animals treated with high fat diet (1 week) then with dose of STZ followed with high fat diet (4 weeks) then treated with Glibenclamide

Orange arrow: normal glomeruli cells

Yellow arrow: no/destroyed glomeruli cells and heavy load of inflammatory cells in kidney

Green arrow: small glomeruli cells and infiltration of inflammatory cells in kidney

Conclusion:

Guggulu consists of oleo-gum resin obtained as an exudates from the tapping of stem and branches of Commiphora wightii (Arnott) Bhandari [syn. Commiphora mukul Engl; Balsamodendron
mukul [Family, Burseraceae]. Guggul exist in five different forms in nature and has been used to treat various disorders. The present study was aimed to developed different formulations and to assess the formulations for their anti-diabetic activity and for other parameters.

The study's objective was to assess the guggul formulations (Kaishore guggul kwatha, Kaishore guggul tablet, triphala guggul kwatha, and Triphala guggul tablet) in STZ-induced diabetes in rats in terms of their ability to reduce blood sugar, lower cholesterol, prevent nephropathy, and increase antioxidant activity. The metabolism and absorption of glucose are both disturbed by diabetes mellitus. By administering kaishore guggul formulations, the elevated plasma glucose levels in diabetic rats were reduced. The potentiation of insulin from the islets of Langerhans'

pre-existing beta cells is what gives kaishore guggul kwatha and kaishore guggul tablet their antihyperglycemic effects.

In order to study the impact on other parameters such as insulin level, total plasma cholesterol, triglyceride, and kidney functioning parameters, the chronic antihyperglycemic model was used along with a high fat diet. These parameters were estimated for all treated groups and compared to a diabetic control group.

The overall results concluded that Kaishore guggul kwatha, Kaishore guggul tablet were found to be effective compared with other formulations and standard.

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