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Nano-suspension of ursolic acid for improving oral bioavailability and attenuation of type II diabetes: A histopathological investigation

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ABSTRACT

Ursolic acid is a pentacyclic carboxylic acid present in medicinal herbs abundantly. There is scientific evidence of important benefits of ursolic acid in metabolism of lipids and glucose, as well as on the body weight and metabolic parameters. However, the hydrophobicity of ursolic acid increases the difficulty in its potential clinical application. The aim of the present study is to develop ursolic acid nanoparticles to enhance its bioavailability and further subjected for evaluating the antidiabetic activity. Ursolic acid nanoparticles were prepared by nanoprecipitation method and optimized by varying formulation variables including PVA concentration and processing variables including stirring speed. Mean Particle Size, Polydispersity index and zeta potential were measured for the optimized nanoparticles. Non-insulin dependent diabetes mellitus was induced intraperitonially in wistar albino rats at 60 mg/kg streptozotocin. Ursolic acid (100 mg/kg) and nanoparticle (UNP 25 mg/kg and 50 mg/kg) were given orally for 4 weeks. *In vivo* antidiabetic effect was estimated by measuring blood glucose level. The Serum cholesterol, triglycerides, SGOT, SGPT, albumin, total protein and *in-vivo* antioxidant parameters were determined by using diagnostic kits. Optimized Ursolic acid nanosuspension showed particle size and polydispersity index to be 246.4 nm and 0.206 respectively. Zeta potential of the prepared nanoparticle was −31.2 ± 5.17 mV. Ursolic acid nanoparticle showed significant reduction ($p < 0.001$) in elevated blood glucose level in dose dependent manner with prominent lipid lowering and antioxidant effect. The promising results from the study revealed that nano-preparation was found to be the most effective for antidiabetic activity due to its changes in oxidative biomarkers.

1. Introduction

Non insulin dependent Diabetes mellitus (NIDDM) is metabolic disorder characterized by the presence of overweight/obesity, inactivity, resistance for insulin, hyperglycemia, dyslipidaemia, negative nitrogen balance and an inflammatory state. It is also described by reduced insulin sensitivity, means, lower ability of insulin to metabolize glucose, a key in the physiopathogeny of disease process (Kaur, 2014). From the several years it has been suggested that patients with diabetes undergo unrelieved oxidative stress. This can be appreciated by measurement of biomarkers for oxidative stress in patients with type II diabetes.

To find out for a therapeutic agent that can emphasis on the metabolic syndrome especially, improve insulin sensitivity to effectively prevention of type II diabetes mellitus, ursolic acid is a promising compound. Ursolic acid [3β-hydroxy-urs-12-ene-28-oic acid] is a ubiquitous pentacyclic triterpenoid in plant kingdom, medicinal herbs, and is an integral part of the human diet has been isolated from many kinds of medicinal plants, such as *Eriobotrya japonica*, *Rosmarinus officinalis*, *Melaleuca leucadendron*, *Ocimum sanctum* and *Glechoma hederaceae*. Literature survey revealed methodical observations of ursolic acid in metabolism of glucose and metabolic factors. Ursolic acid acts as insulin secretagogue and the glucose imbalance is maintained by modulation of calcium and protein kinase C as discussed by Castro et al. (2015). It ameliorates glucose metabolism primarily by the activation of peroxisome proliferator-activated receptor alpha and induction of the hepatic autophagy pathway as shown by Jia et al. (2015). It also increases glucose uptake through the PI3K signaling pathway in

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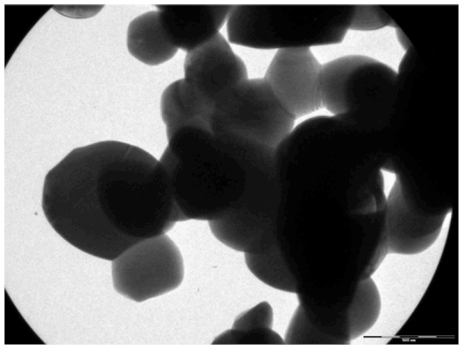

Fig. 1. High Resolution Transmission Electron Micrograph of Ursolic acid Nanosuspension.

adipocytes presented by He et al. (2014). Ursolic acid also exerts a protective effect on kidney, liver by a method of Biswas et al. (2019) and effective in the treatment of diabetic nephropathy as demonstrated by Ling et al. (2013) It is a potent antioxidant with hydrophobic nature which increases the difficulty in its potential clinical applications. In present study we have made an attempt to prepare ursolic acid nanoparticles for enhancing the bioavailability and further subjected for evaluating the in-vivo antidiabetic activity.

2. Material and methods

2.1. Chemicals

The standard drug ursolic acid (otto chemika-biochemika reagents), technical grade Streptozotocin (purity 99.4%) (Cal Biochem, Mumbai) and Standard drug Metformin (CDH, India) were used. For measuring the biochemical and serum parameters the diagnostic kits (Erba Pharmaceuticals, New Delhi) were used. All the reagents and chemicals used were of AR grade.

2.2. Preparation of nanoparticles of ursolic acid

Nano-precipitation technique was used to prepare Ursolic acid nanoparticles (UANs) with slight change of a previously reported method of Mishra et al. (2013). Briefly, 2 g of UA was dissolved in 25 ml of ethanol and acetone (1:3) by ultra-sonication at 40 W for 15 s. The resulting solution was then injected (1 ml/min) with a micro syringe connected to a thin Teflon tube, into 60 ml distilled water containing 2% w/v of polyvinyl alcohol (PVA) with continuous stirring at 500 rpm. The resulting emulsion obtained was then diluted with 120 ml PVA solution (0.3% w/v in water) in order to minimize coalescence and the mixture was continuously stirred (500 rpm) for 12 h at room temperature to allow evaporation of solvent and nanoparticles formation. The resulting Ursolic acid nanoparticle was subsequently cooled down to −20° C and freeze dried.

2.3. Morphological analysis and characterization of nanoparticle

About 100 μL of the prepared nanoparticles was diluted to 5 ml of distilled water and analysed with dynamic light scattering Zetasizer (Malvern Zetasizer, UK). The analysis was carried out at 25 °C. Morphological analysis of nanoparticles was performed by High Resolution Transmission Electron Microscopy (HRTEM) by method of Clogston and Patri (2011); Cao et al. (2016). FTIR spectrum of Ursolic acid, PVA and nanoparticle were recorded by placing the sample on KBr Plate. The mixtures were subjected for FTIR measurements over the range of 650–4000 cm^{-1}(PerkinElmer Spectrum version 10.03.02, USA).

2.4. Optimization of nanosuspension

Formulation variables that were varied for preparing nanoparticle include concentration of PVA and ethanol: acetone ratio and process variable include stirring speed. For optimization, the concentration of PVA was varied (1.5%, 2.0%, 2.5%, 3.0%) along with the stirring speed (250 rpm, 500 rpm, 750 rpm, 1000 rpm) but keeping ethanol: acetone ratio constant.

On varying ethanol: acetone ratio (1:1, 1:2, 1:3, 1:4) and concentration of PVA (1.5%, 2.0%, 2.5%, 3.0%) but keeping stirring speed constant (500 rpm), 32 formulations have been prepared and optimized on the basis of Particle size, pdI and Zeta potential.

2.5. Pharmacological studies

2.5.1. Experimental animals

Young adult male albino wistar rats 6–8 weeks old, weighing 200–250 g were kept in the animal facility of United Institute of Pharmacy, Allahabad. The animals were kept in polypropylene cages in standard environmental conditions, 12 h light and 12 h dark cycle at 25 ± 2 °C. Before and during the experiments, the rats were fed with standard laboratory pellet diet and water *ad libitum*. Animals were acclimatized to the laboratory condition for at least 5 days prior to the experiment and were maintained in a well-ventilated animal house. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) with approval number (REG. No: UIP/IAEC/

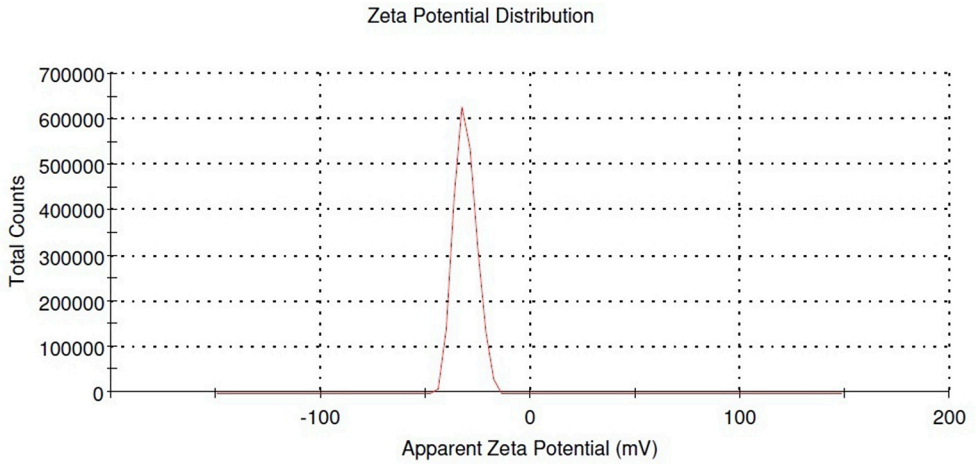

Fig. 2. Zeta Potential of Ursolic acid Nanoparticles.

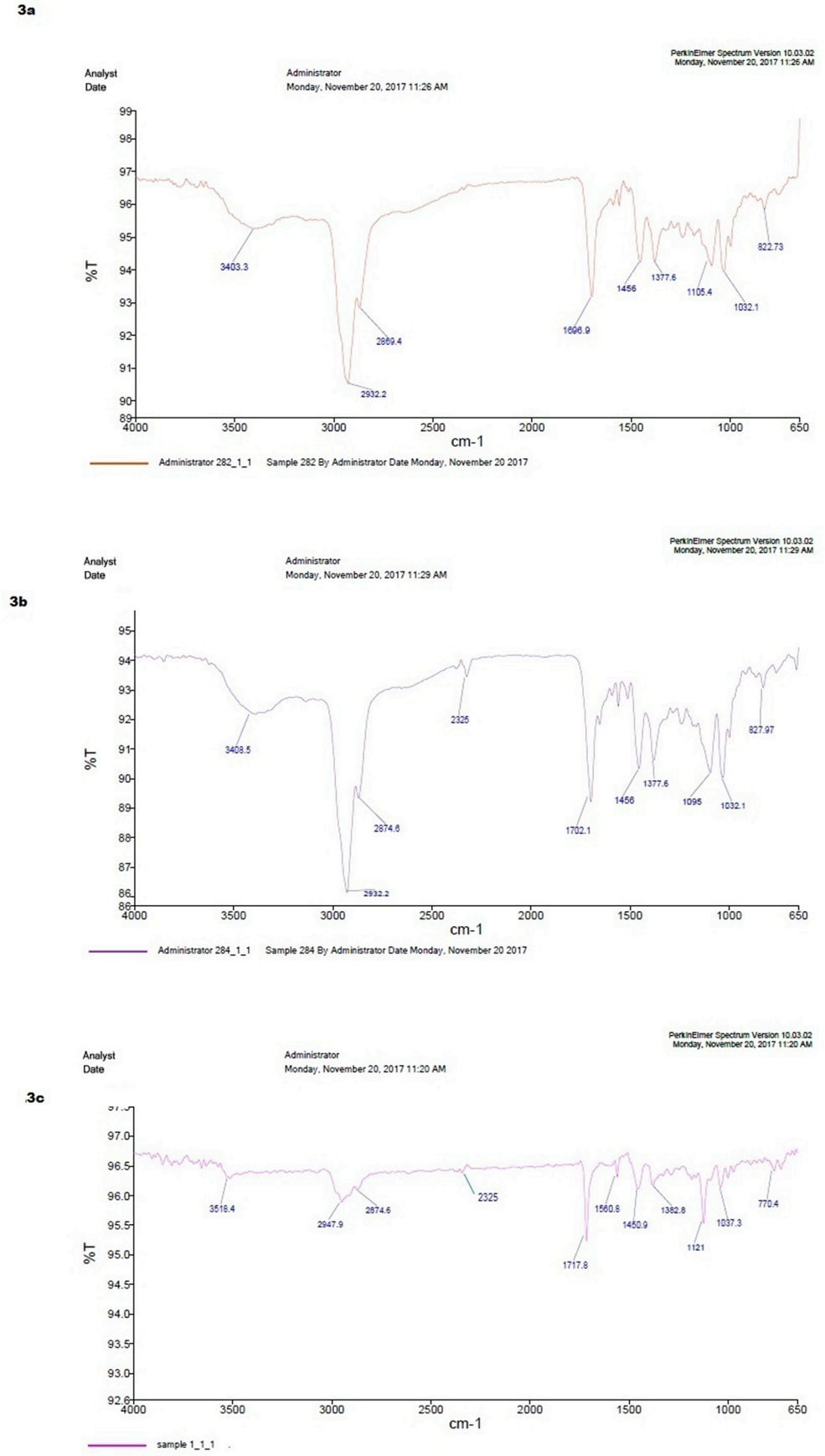

Fig. 3. FTIR Spectra of Ursolic acid, PVA and ursolic acid nanoparticles.

Table 1
Optimization of Ursolic acid Nanosuspension by varying PVA concentration (%) and Stirring speed (rpm).

Formulation	PVA (%)	Stirring speed (rpm)	Particle size (nm) ± SD[a]	pdI ± SD[a]	Zeta potential (mV) ± SD[a]
F1	1.5	250	500.9 ± 4.5	0.288 ± 0.02	18.6 ± 1.2
F2	1.5	500	307.3 ± 3.2	0.159 ± 0.05	29.8 ± 1.5
F3	1.5	750	431.8 ± 2.4	0.225 ± 0.11	15.7 ± 1.8
F4	1.5	1000	424.7 ± 1.5	0.274 ± 0.13	12.7 ± 1.0
F5	2.0	250	489.3 ± 3.5	0.268 ± 0.03	17.4 ± 1.7
F6	2.0	500	288.9 ± 3.0	0.188 ± 0.15	31.8 ± 2.0
F7	2.0	750	467.7 ± 2.0	0.241 ± 0.04	20.8 ± 1.4
F8	2.0	1000	410.3 ± 1.1	0.296 ± 0.16	17.9 ± 0.09
F9	2.5	250	499.8 ± 1.7	0.356 ± 0.12	10.6 ± 1.9
F10	2.5	500	325.3 ± 1.3	0.171 ± 0.06	30.9 ± 2.2
F11	2.5	750	432.7 ± 2.2	0.285 ± 0.08	19.7 ± 2.4
F12	2.5	1000	478.5 ± 2.8	0.315 ± 0.17	16.8 ± 2.5
F13	3.0	250	512.7 ± 0.8	0.321 ± 0.09	15.8 ± 1.1
F14	3.0	500	364.6 ± 3.8	0.194 ± 0.10	28.9 ± 2.6
F15	3.0	750	498.7 ± 4.0	0.336 ± 0.14	14.8 ± 2.8
F16	3.0	1000	500.8 ± 4.2	0.359 ± 0.07	13.7 ± 2.0

[a] The data represents as mean ± Standard deviation. (n = 3).

Table 2
Ursolic acid Nanosuspension prepared by varying PVA Concentration and Ethanol: Acetone ratio (v/v).

Formulation	PVA (%)	Ethanol: Acetone ratio (v/v)	Particle size (nm) ± SD[a]	pdI ± SD[a]	Zetapotential (mV) ± SD[a]
F17	1.5	250	355.9 ± 3.5	0.092 ± 0.06	19.5 ± 2.2
F18	1.5	500	350.7 ± 3.2	0.159 ± 0.08	21.9 ± 2.5
F19	1.5	750	268.6 ± 1.4	0.186 ± 0.15	24.7 ± 1.5
F20	1.5	1000	350.3 ± 2.5	0.228 ± 0.04	26.4 ± 1.1
F21	2.0	250	325.9 ± 2.7	0.214 ± 0.23	30.8 ± 1.7
F22	2.0	500	276.9 ± 1.1	0.225 ± 0.19	28.2 ± 2.4
F23	2.0	750	246.2 ± 1.3	0.206 ± 0.14	31.2 ± 1.4
F24	2.0	1000	301.8 ± 1.7	0.220 ± 0.21	27.9 ± 1.3
F25	2.5	250	335.8 ± 4.7	0.231 ± 0.20	22.6 ± 1.8
F26	2.5	500	398.4 ± 4.3	0.221 ± 0.17	20.9 ± 2.9
F27	2.5	750	296.5 ± 2.8	0.254 ± 0.11	18.5 ± 0.09
F28	2.5	1000	312.2 ± 3.8	0.248 ± 0.12	19.9 ± 2.8
F29	3.0	250	330.2 ± 1.5	0.211 ± 0.09	17.6 ± 1.1
F30	3.0	500	344.6 ± 2.9	0.242 ± 0.10	16.7 ± 2.6
F31	3.0	750	325.4 ± 4.0	0.235 ± 0.16	15.9 ± 2.1
F32	3.0	1000	293.2 ± 4.2	0.230 ± 0.05	14.9 ± 2.0

[a] The data represents as mean ± Standard deviation. (n = 3).

April-2015/07). The care of animals and the laboratory was carried out as per the CPCSEA regulation.

2.5.2. Selection of dose for antidiabetic activity

As a small natural molecule universally present in plants and even human diet; Ursolic acid is relatively nontoxic and is well tolerated orally and topically in both human and rodents. The acute toxicity (LD_{50}) of ursolic acid in rodents was determined by Lee et al. (2010) and was found to be 8330 mg/kg for oral administration and more than 637 mg/kg for intraperitoneal injections as discussed by Xu et al. (2011). Therefore, the dose selected for the pharmacological activity of ursolic acid is 100 mg/kg and its nanoparticle 25 mg/kg and 50 mg/kg were selected as per Yang et al. (2012).

2.5.3. Experimental induction of diabetes

All the animals were kept at overnight fasting and the diabetes was induced intraperitonially with streptozotocin 60 mg/kg. Rats having fasting blood glucose level above 200 mg/dl was considered to be hyperglycemic and used further for activity.

2.5.4. Experimental design

The animals were divided in six different groups (n = 6). The standard drug along with the nanoparticle was given orally to rats by oral gavage.

Following were the groups used for the activity:

Group I: positive control received vehicle normal saline solution
Group II: diabetic control induced with streptozotocin (60 mg/kg) intraperitonially
Group III: diabetic rats treated with drug Ursolic acid (100 mg/kg)
Group IV: diabetic rats treated with 25 mg/kg UNP
Group V: diabetic rats treated with 50 mg/kg UNP
Group VI: diabetic rats treated with standard drug Metformin 100 mg/kg

The drugs and vehicles orally administered through oral gavage tube daily for four weeks. Blood was withdrawn from the retro orbital puncture of rats on 0th day, 7th day, 14th day, 21st day and 28th day to measure blood glucose level. Blood glucose level was determined by one touch glucometer (Jonhson & Johnson, Mumbai). Biochemical parameters, total protein, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), albumin level, cholesterol and triglycerides were also been estimated by using diagnostic kits.

2.5.5. In-vivo antioxidant activity in diabetic rats

Measurement of Catalase (CAT), Superoxide dismutase (SOD), Glutathione -S-transferase (GST), Reduced glutathione (GSH) and Glutathione peroxidase (GPx) were determined for ascertain its antioxidant activity by standard procedure (Murugan and Pari, 2006).

2.5.6. Histopathological assessment

All tissue specimens were obtained from the same region of the right lobe of the liver, right kidney and Pancreas which were fixed in 10% buffered formalin, processed for embedding in paraffin wax by routine protocols and 5 μm-thick sections were then cut by microtome. The sections were stained with haematoxylin/eosin using a routine protocol and examined using an Olympus BX50 photomicroscope at 250X by a method of Attalla et al. (2010). The pathological findings of examination using light microscopy were recorded.

3. Results

3.1. Characterization and morphological analysis of nanoparticles

The mean particle size and polydispersity index of prepared nanoparticle was found to be 246.4 ± 4.21 nm and 0.206 respectively. The small particle size and shape confirmed by HR-TEM in Fig. 1, which shows more uptake of drug and increases bioavailability of the prepared nanoparticle. Zeta potential of the prepared nanoparticle was −31.2 ± 5.17 mV as shown in Fig. 2. Fourier Transform Infrared Spectra of Ursolic acid, Poly Vinyl Alcohol and ursolic acid nanosuspension were shown in Fig. 3. Prominent peaks were seen at 2325 cm^{-1} for ursolic acid standard in Fig. 3a] and its nanosparticles in Fig. 3b which were remained unaffected by the presence of polymer (PVA) in Fig. 3c.

3.2. Optimization of nanosuspension

A 3D surface graph was plotted using Microsoft excel (2003) software using four levels. About 16 formulations have been prepared by varying PVA concentration and stirring speed but keeping ethanol: acetone ratio (1:1) constant as shown in (Table 1).

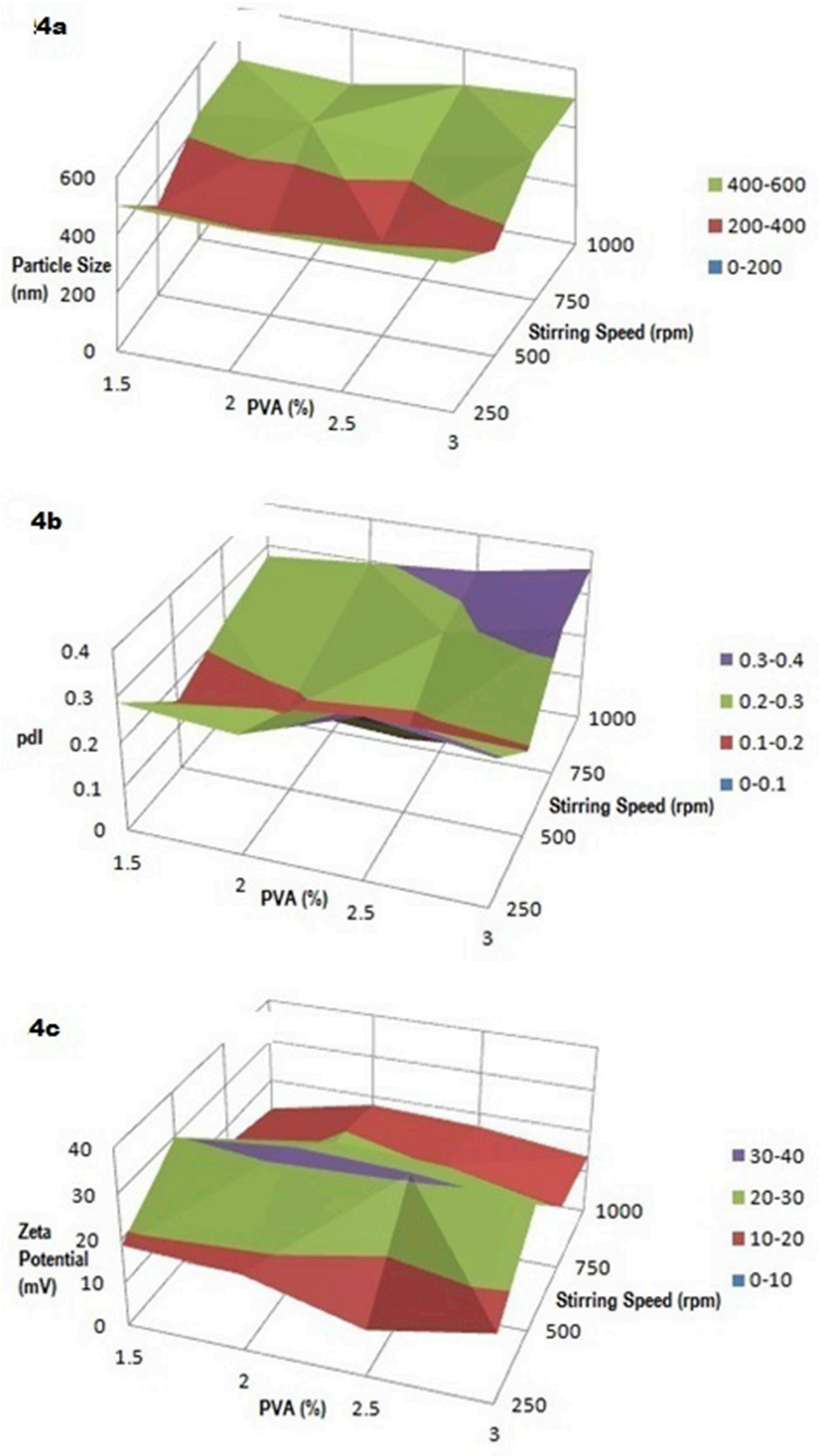

Fig. 4. Optimization of Ursolic acid Nanoformulation by varying PVA Concentration (%) and Stirring Speed (rpm).

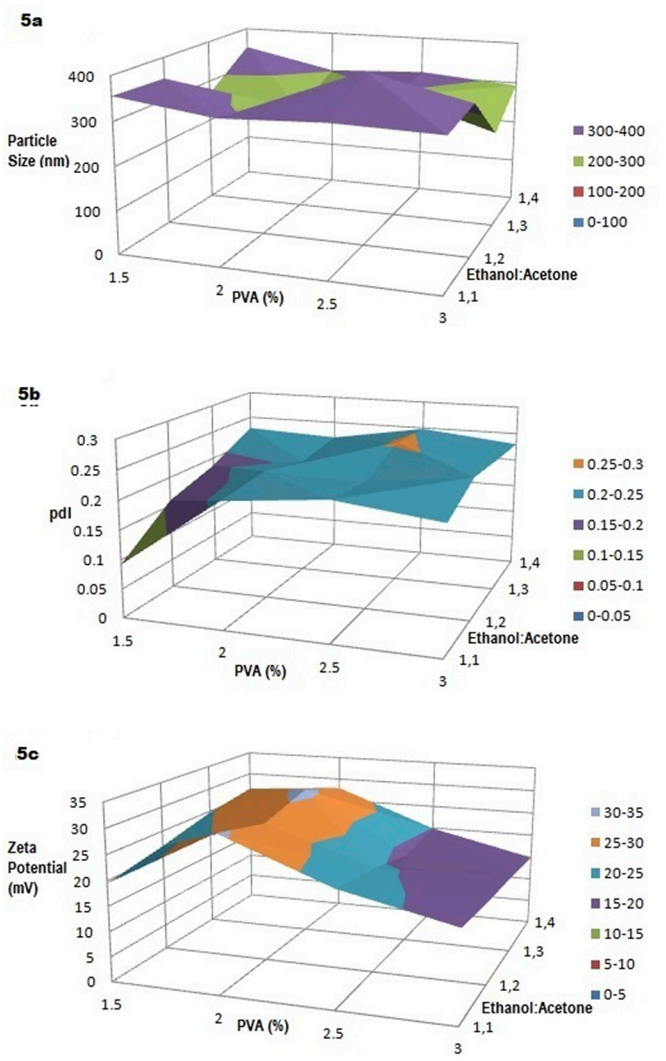

Fig. 5. Optimization of Ursolic acid Nanoformulation by varying PVA Concentration (%) and Ethanol: Acetone ratio (v/v).

Furthermore, by varying PVA concentration and ethanol: acetone ratio but keeping stirring speed constant (500 rpm) 16 formulations been prepared as shown in (Table 2).

PVA at different concentrations including 1.5%, 2%, 2.5% and 3% have been used as a stabilizer and polymer for preparing nanosuspension. Different stirring speed at 250 rpm, 500 rpm, 750 rpm and 1000 rpm were also tried for the preparation. Fig. 4 shows that on increasing the concentration of PVA from 1.5% to 2% and stirring speed from 250 rpm to 500 rpm the particle size of nanosuspension in Fig. 4a and pdI Fig. 4b was decreasing but Zeta potential was increasing in Fig. 4c when compared with an increased particle size on increasing the concentration of PVA above 2% and stirring speed above 500 rpm.

Various combinations of ethanol: acetone (1:1, 1:2, 1:3, 1:4) as solvent and PVA (1.5%, 2.0%, 2.5% and 3%) have been tried for the preparation of ursolic acid nanosuspension. Ethanol and acetone both are less toxic and also produces suitable nanoparticles on precipitation. From the studies, the effect on particle sizes has been observed at different solvent ratios and was found that at 2% PVA and ethanol: acetone (1:3) ratio had produced optimum and uniform sized nanoparticles as shown in Fig. 5. On decreasing the solvent ratio and at lower concentration of PVA, the particle size and pdI were increased but zeta potential were decreased but on increasing the solvent ratio and at intermediate level of PVA the particle size in Fig. 5a and pdI in Fig. 5b decreases but zeta potential in Fig. 5c was increased. F23 formulation was found to be optimum and hence selected for the activity.

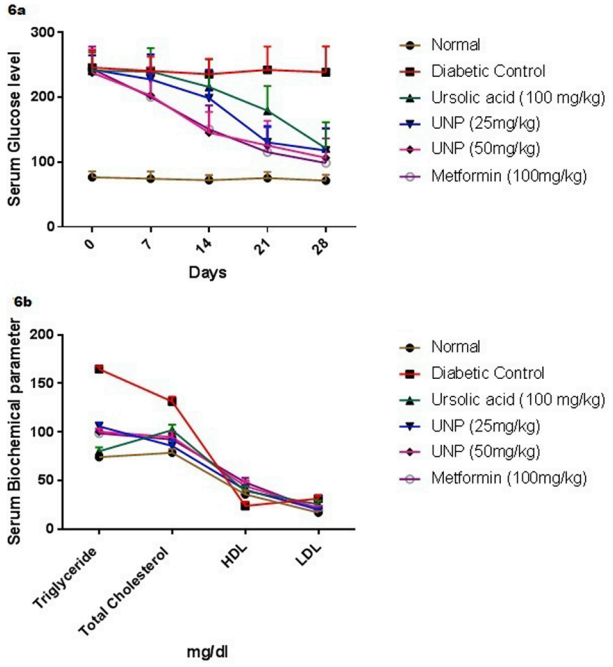

Fig. 6. Representation of serum glucose level and biochemical parameters on experimental animals after 28 days of treatment.

Table: 3

Effect of oral administration of Ursolic acid nanosuspension in Streptozotocin (60 mg/kg) induced diabetic rats on serum SGOT, SGPT, total protein and albumin.

Groups	SGPT (mg/dl) ±SD*	SGOT (mg/dl) ±SD*	Total Protein (mg/ml) ± SD	Albumin (g/dl) ±SD*
Group I	26.18 ± 4.08	68.99 ± 10.33	7.11 ± 1.78	4.86 ± 2.18
Group II	95.01 ± 5.04z†	105.11 ± 11.11^{z}	5.41 ± 1.98^{z}	3.30 ± 1.87^{z}
Group III	75.92 ± 5.71	84.44 ± 13.77	5.11 ± 2.33	3.16 ± 1.39
Group IV	50.71 ± 4.92a	63.11 ± 12.33^{a}	6.07 ± 2.22^{a}	4.40 ± 2.77^{a}
Group V	43.10 ± 5.92c	42.03 ± 15.44^{c}	6.24 ± 3.02^{b}	4.52 ± 2.54^{b}
Group VI	35.62 ± 4.95c	38.92 ± 9.55^{c}	6.29 ± 2.87^{b}	4.57 ± 2.66^{b}

*The data represents as mean ± Standard deviation of six rats in each group.†$^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ compared to diabetic control group. $^{z}P < 0.001$ as compared to control group.

3.3. *In vivo antidiabetic studies*

The effect of oral daily administration of ursolic acid (100 mg/kg), UNP (25 mg/kg) and UNP (50 mg/kg) and metformin (100 mg/kg) was shown in Fig. 6a at the end of the treatment. Blood glucose levels of standard drug ursolic acid (100 mg/kg) were reduced from 238.67 ± 39.76 to 121.21 ± 40.34, $p < 0.001$, UNP (25 mg/kg) were reduced from 238.67 ± 39.76 to 117.78 ± 33.76, $p < 0.001$, UNP (50 mg/kg) were reduced from 238.67 ± 39.76 to 106.65 ± 45.98, $p < 0.001$ and metformin (100 mg/kg) were reduced from 238.67 ± 39.76 to 98.65 ± 37.87, $p < 0.001$.

Table 4
The Effect of Nano-suspension on *in vivo* antioxidant activity.

Parameters	GSH (nM of DTNB conjugated/mg protein)	GST (μmol of CDNB-GSH conjugate formed/min/mg protein)	GPx (μg glutathione consumed/(min/mg protein)	CAT (μmol of H_2O_2 consumed/min/mg protein)	SOD (U min/(mg/Hb in erythrocytes)
Normal control (Group I)					
Liver	125.97 ± 2.56	8.47 ± 0.77	10.53 ± 0.81	75.28 ± 2.57	6.34 ± 0.16
Kidney	115.87 ± 1.31	7.88 ± 0.64	5.73 ± 0.18	37.39 ± 1.52	
Diabetic control (Group II)					
Liver	70.13 ± 1.65^{z†}	4.23 ± 0.28^{z}	6.81 ± 0.78^{z}	37.76 ± 1.92^{z}	3.89 ± 0.25^{z}
Kidney	43.97 ± 2.43^{y}	3.66 ± 0.17^{y}	3.79 ± 1.01^{y}	22.45 ± 1.65^{y}	
Ursolic acid (100 mg/kg) (Group III)					
Liver	115.95 ± 4.89^{b}	5.25 ± 0.44^{b}	7.14 ± 0.59^{b}	60.96 ± 3.19^{c}	5.99 ± 0.44^{b}
Kidney	90.02 ± 3.99	4.00 ± 0.49^{a}	5.21 ± 0.32^{b}	25.54 ± 1.56^{b}	
UNP (25 mg/kg) (Group IV)					
Liver	120.67 ± 3.04^{c}	7.95 ± 0.35^{c}	8.87 ± 0.69^{c}	65.12 ± 1.74^{c}	6.01 ± 0.38^{c}
Kidney	88.63 ± 3.89	5.31 ± 0.55^{b}	6.78 ± 0.78^{c}	31.32 ± 1.21^{c}	
UNP (50 mg/kg) (Group V)					
Liver	124.62 ± 3.16^{c}	7.12 ± 0.49^{c}	9.55 ± 0.88^{c}	72.32 ± 3.18^{c}	6.46 ± 0.49^{c}
Kidney	102.94 ± 1.56^{b}	6.05 ± 0.76^{c}	5.55 ± 0.79^{c}	36.18 ± 2.52^{c}	
Metformin (100 mg/kg) (Group VI)					
Liver	125.96 ± 1.96^{c}	6.13 ± 0.34^{c}	7.02 ± 0.55^{b}	67.67 ± 3.59^{c}	5.55 ± 0.21^{b}
Kidney	96.27 ± 3.57	3.00 ± 0.89^{a}	4.66 ± 0.78^{a}	34.95 ± 1.61^{c}	

The data represents as mean ± Standard deviation of six rats in each group.†$^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ compared to diabetic control group.$^{z}P < 0.001$ as compared to control group.

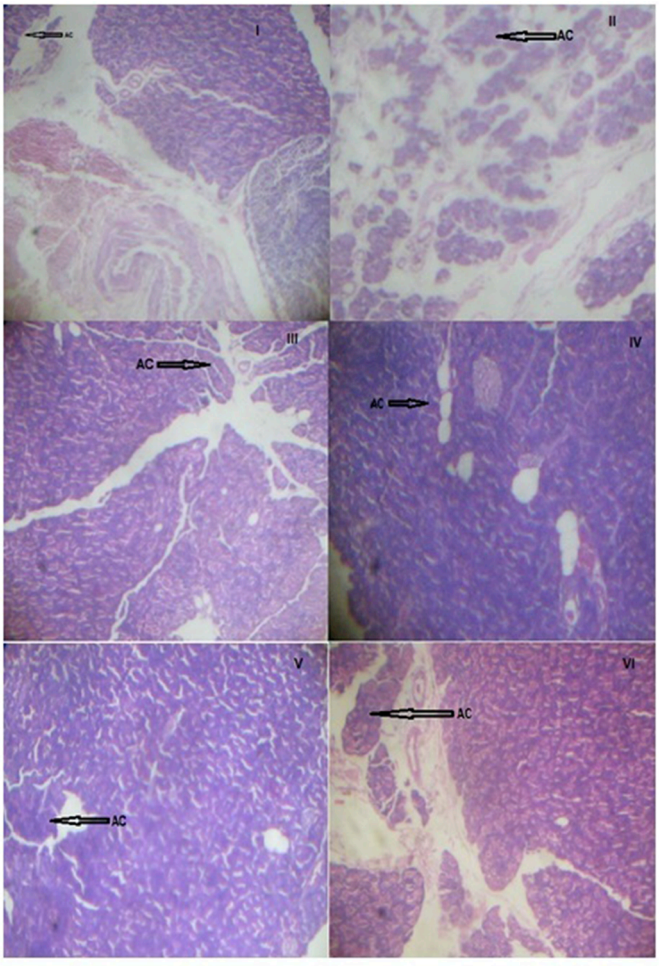

Fig. 7. Histopathological Assessment of Portion of Pancreas. I- Normal rats, II-Diabetic Control, III-Ursolic acid (100 mg/kg), IV-UANP (25 mg/kg), V- UANP (50 mg/kg), VI- Metformin (100 mg/kg). AC-Acinar Cells.

3.4. Biochemical parameters

The effect of Ursolic acid nanoparticles on biochemical parameters including triglyceride level, total cholesterol level, HDL level and LDL level for all the groups were shown in Fig. 6b. The triglyceride, total cholesterol, HDL and LDL levels for group I are 74.32 ± 1.92, 78.79 ± 3.72, 35.95 ± 4.17, 17.26 ± 1.52 respectively. There was significant reduction in triglyceride level in Group III (164.8 ± 2.83–80.22 ± 4.01, $p < 0.001$), Group IV (164.8 ± 2.83–105.98 ± 3.72, $p < 0.001$), Group V (164.8 ± 2.83–100.35 ± 3.27, $p < 0.001$), Group VI (164.8 ± 2.83–98.67 ± 2.78, $p < 0.001$), in total cholesterol in Group III (131.41 ± 4.85–102.11 ± 5.53, $p < 0.01$), Group IV (131.41 ± 4.85–85.79 ± 6.06, $p < 0.001$), Group V (131.41 ± 4.85–94.98 ± 5.41, $p < 0.001$), Group VI (131.41 ± 4.85–92.45 ± 4.98, $p < 0.001$), in LDL level in Group III (31.61 ± 2.98–25.74 ± 3.74, $p < 0.01$), Group IV (31.61 ± 2.98–20.40 ± 1.23, $p < 0.001$), Group V (31.61 ± 2.98–22.14 ± 2.15, $p < 0.001$), Group VI (31.61 ± 2.98–19.65 ± 3.96, $p < 0.001$) but HDL levels are significantly increased in Group III (24.07 ± 4.40–39.47 ± 4.93, $p < 0.001$), Group IV (24.07 ± 4.40–40.54 ± 4.54, $p < 0.001$), Group V (24.07 ± 4.40–44.35 ± 5.47, $p < 0.001$), Group VI(24.07 ± 4.40–48.33 ± 4.71, $p < 0.001$) as compared with diabetic control group.

(Table 3) shows outcome of oral administration of standard drug, ursolic acid nano particles and metformin on serum SGOT, SGPT, total protein and albumin on 28th day of the study. Serum SGPT level was significantly decreased in Group V (95.01 ± 5.04–43.10 ± 5.92, $p < 0.001$) and Group VI (95.01 ± 5.04–35.62 ± 4.95, $p < 0.001$) as compared with Group III (95.01 ± 5.04–75.92 ± 5.71) and Group IV (95.01 ± 5.04–50.71 ± 4.92, $p < 0.05$). The levels of serum SGOT was much reduced in Group V (105.11 ± 11.11–42.03 ± 15.44, $p < 0.001$) and Group VI (105.11 ± 11.11–38.92 ± 9.55, $p < 0.001$) as compared to Group IV (105.11 ± 11.11–63.11 ± 12.33, $p < 0.05$) and Group III (105.11 ± 11.11–84.44 ± 13.77). Significant decrease in total protein ($p < 0.01$) and albumin ($p < 0.01$) were observed for all the treated groups.

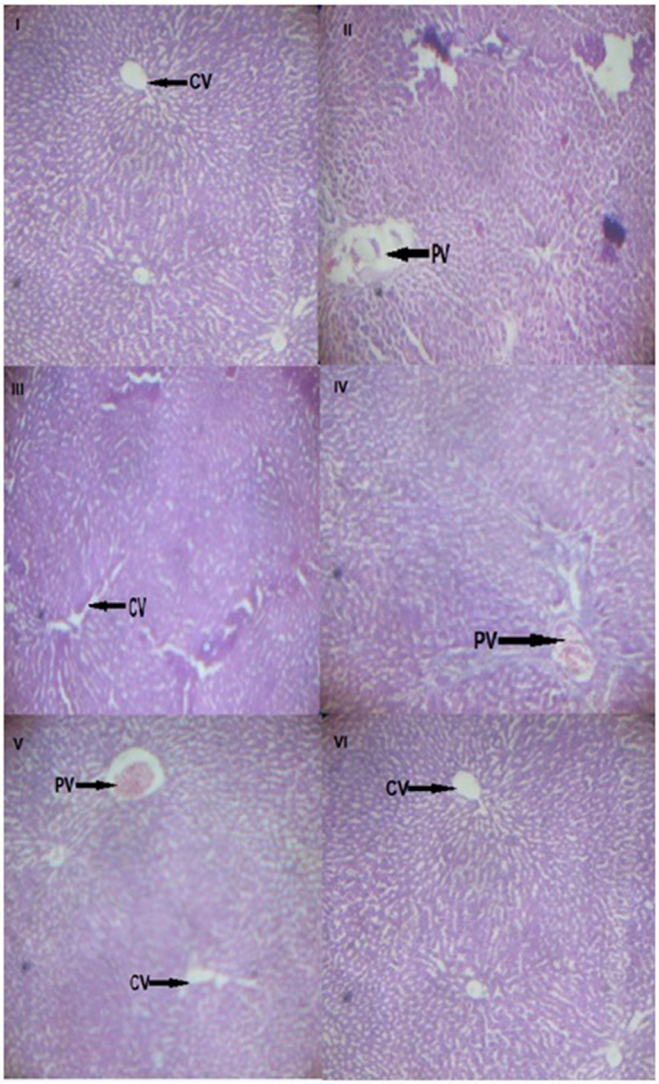

Fig. 8. Histopathological Analysis of Portion of Liver. I- Normal rats, II-Diabetic Control, III-Ursolic acid (100 mg/kg), IV-UANP (25 mg/kg), V- UANP (50 mg/kg), VI- Metformin (100 mg/kg). CV-Central Vein, PV-Portal Triad.

3.5. In vivo antioxidant activities

The effect of UAN on antioxidant status, the activities of enzymatic antioxidants SOD, CAT, GPx and GST and non-enzymatic antioxidant GSH were estimated and results were shown in (Table 4). The activities of enzymatic and levels of non-enzymatic antioxidant were significantly decreased in diabetic control rats. After 28 days treatment with UAN 50 mg/kg, there is significant increase in GST and CAT level in liver 4.23 to 7.12 and 37.76 to 72.32 respectively ($p < 0.001$), The SOD level (3.89 U min/mg Hb) in erythrocytes decreased on the induction of diabetes, but significantly increased on treatment with UAN, thereby restoring the SOD level to nearly that of the normal control group. The results were comparable to that of Metformin. In comparison to the normal control rats, the activities of these enzymes decreased significantly in the diabetic control rats. The oral administration of UAN for 4 weeks significantly reversed these enzymes to near-normal values.

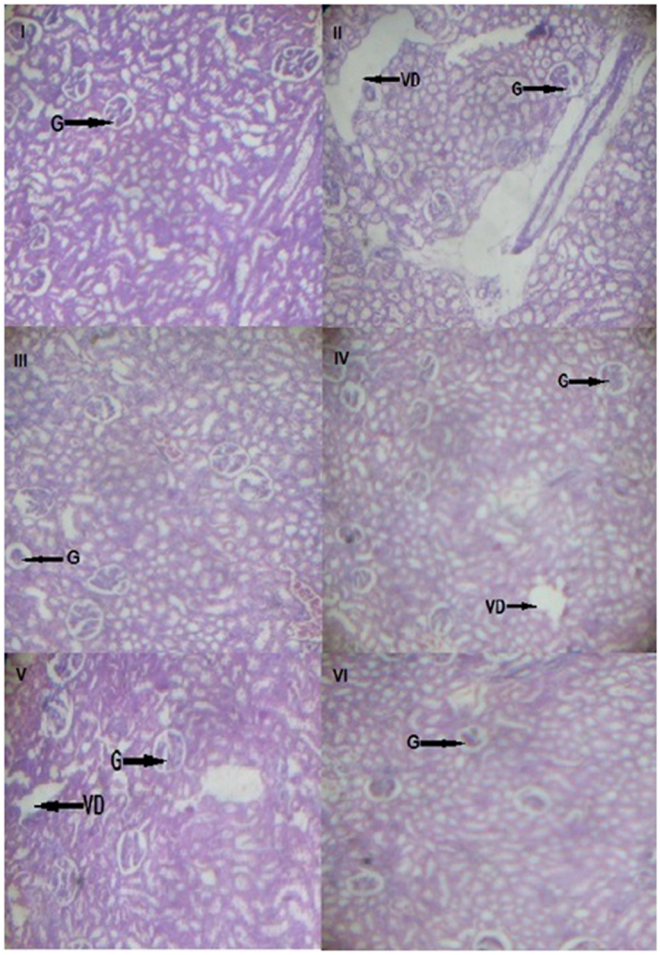

Fig. 9. Histopathological estimation of Portion of Kidney. I- Normal rats, II-Diabetic Control, III-Ursolic acid (100 mg/kg), IV-UANP (25 mg/kg), V- UANP (50 mg/kg), VI- Metformin (100 mg/kg). VD-Vacuolar degeneration, G-Glomerulus.

3.6. Histopathological assessment of pancreas, liver & kidney

3.6.1. Pancreas

In Fig. 7, Group I showed uniformly distributed nuclei with cells. STZ (60 mg/kg) induced diabetic rats shows necrosis of pancreatic islets and lymphocytic infiltrates. Metformin (100 mg/kg) treated rats and standard Ursolic acid treated rats showed reduced infiltration with necrosis. UNP 25 mg/kg & UNP 50 mg/kg showed normal architecture with no lymphocytic infiltration.

3.6.2. Liver

In Fig. 8, Group I showed central vein with normal portal triad along with many hepatocytes. STZ (60 mg/kg) induced diabetic rats showed distorted portal triad with numerous cellular infiltrates. Group III showed reduced necrosis with restored portal triad and reduced congestion of central vein. Group IV showed mild inflammatory lymphocytes with patchy necrosis. UNP (50 mg/kg) treated rats showed mild hemorrhage with normal central vein and numerous hepatocytes. On treatment with the standard drug metformin (100 mg/kg) restoration of architecture was observed as with the normal control.

3.6.3. Kidney

In Fig. 9, Group I showed normal architecture with distinct tubules stroma. Group II showed presence of severe vacuolar degeneration of tubules, inflammatory cells with increased glomerular space. Group III

showed reduced tubular degeneration with inflammatory cells with increased glomerular space. Group IV showed mild degeneration of tubules and infiltration of inflammatory cells. Group V showed distinctive tubules with normal glomerular size. Group VI showed less infiltration and restoration of architecture as of normal control.

4. Discussion

Scientific reports established technologies to improve solubility of food derived bioactive compounds by Recharla et al. (2017). Nanoprecipitation technique for preparing ursolic acid nanoformulation proves scientific approach towards enhancement of bioavailability using ethanol as a solvent and PVA as a suitable stabilizer. In 32 formulations, the critical variables, such as the concentration of PVA, solvent ratio and stirring speed significantly affect the physicochemical properties and stability profiles of the produced nanosuspension. The resultant nanoparticle with smaller mean particle size confirmed an optimized formulation as evidenced by TEM. The values of PDI ranged between 0.189 and 0.662 were legitimate to the heterogeneity thereby considered as a factor to characterize nanoparticle and to study surface modifications as shown by Clayton et al. (2016). The prepared formulation is homogeneous as it has better PdI values. FTIR spectra of the formulation showed the peaks in 650 cm^{-1} to 4000 cm^{-1} region with no suppression of peaks and hence overcome the possibilities of drug polymer interactions.

Pentacyclic triterpenoid are widely distributed in food and plants. Traditionally it is used in the treatment of diabetic and diabetic complications as discussed by Alqahtani et al. (2013). In this study ursolic acid nanoparticle showed that serum glucose levels can be decreased effectively in a dose dependant manner. Ursolic acid stimulates the glucose uptake through the involvement of the classical insulin signaling related to the GLUT4 translocation to the plasma membrane as well as the GLUT4 synthesis by Castro et al. (2015).

Increased lipid in the liver and triglycerides impairs the ability of insulin to regulate gluconeogenesis and glycogen synthesis. This impaired insulin action will lead to insulin resistance which eventually results in pancreatic β-cell failure and the circulating insulin levels become insufficient to control blood glucose level, leading to overt hyperglycemia by demonstrated by Cooney et al. (2002); (Samuel and Shulman, 2012). UA nanoparticle had decreased the levels of triglycerides and increase the level of HDL in dose dependant manner thereby improves the action of insulin.

In this study diabetes was induced by Streptozotocin as it induces pancreatic swelling and causes degeneration in islets of Langerhans β cells and induces experimental diabetes as discussed by Kaur et al. (2017). The possible mechanism of metformin is it increases the peripheral uptake of glucose in the presence of insulin. Ursolic acid nanoparticle has curative action on pancreatic cytotoxicity and hyperglycemia by reducing elevated oxidative stress.

Serum SGPT and SGOT are the blood test which indicates liver damage and nephropathy is an indicative sign of diabetes. The raised serum levels in diabetic control group were significantly decreased on the treatment of diabetic rats with ursolic acid nanoparticles since these are involved in protection of β-cells as discussed by Jang et al. (2009). The levels of total protein and serum albumin increased in diabetic treated rats with ursolic acid nanoparticles primarily at 50 mg/kg dose showed the effect of nanoparticles in dose dependant manner. Moreover, the increase in the albumin level in rats treated with UNP 50 mg/kg indicate retention of albumin in blood which improves renal complications associated with diabetes.

Diabetic induction with STZ (60 mg/kg) had exaggerated the condition of organs. The histopathological assessment of organs showed ameliorated condition of organs upon treatment with UNP in dose dependent manner.

5. Conclusion

In conclusion, we found that an oral dose of UNP 50 mg/kg exhibit therapeutic effect against diabetes. Thus, we suggest that the nanoparticles system can be applied to overcome other water poorly soluble natural constituents and furthermore to decrease the treatment dosage. This study could serve as a suitable reference to permit the future utilization of nanoparticulate system as a novel preventive and therapeutic measure for the treatment of diabetes and its related metabolic complications.

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Declaration of competing interest

The authors declare no conflict of interest.

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