Evaluating the antidiabetic effect of Turmeric extract on streptozotocin induced Diabetic rats

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Abstract: Diabetes is a major risk factor for coronary artery diseases, nephropathy, neuropathy, retinopathy and many other complications. Turmeric and its bioactive compounds like curcumin had great therapeutic abilities against diabetes. In this study turmeric and its extract was evaluated for its hypoglycemic potential in streptozotocin induced hyperglycemic rats for thirty days. For this purpose, turmeric extract obtained was analyzed for its antioxidant potential via screening tests like DPPH, TPC. The best result for TPC was seen with concentration of 70% of ethanolic extract gives the best result for TPC and its total phenolic content was 536.56 ± 2.24 mg GAE/100mg followed by methanolic extract at the concentration of 70% gives the TPC value 529.62 ±6.56 GAE/100mg and from acetone the best extraction percentage was also 70 % and its TPC value was 524.94 ± 1.54. The maximum DPPH value was seen with ethanolic extract at concentration of 70% 59.58 ±2.89 At the end of the study turmeric extracts administrated rats was kept fasted overnight and then it was analyzed for their glucose, insulin. For insulin the maximum effect was seen with ethanolic extract 11.30b±0.04. The ethanolic extract at the concentration of 70% cause maximum decrease in blood glucose level.

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Key words: TPC (total phenolic contents), Thiobarbituric acid reactive substances (TBARS), Diabetes.

1. Introduction:

Vegetables and fruits have gained much importance in diet because they are rich in vitamins, especially vitamin C, vitamin A, phytochemicals including antioxidants, fibers and minerals. Fruits and vegetables have higher amount of nutrients and provide potassium etc. Higher fruit and vegetables intake protective against several degenerative diseases like cancer, atherosclerosis (Hornick *et al*., 2011). Turmeric also called *Curcuma longa,* its family is Zinberaceae, production regions of turmeric in the tropical areas of South Asia, Pakistan, India, China and Bangladaish. The second largest producer of turmeric is Pakistan. It produces in Meerpur Khas, Kassor, Okara, Bannu and Lahore. Out of total production of turmeric more than 80% produce in Kasoor (Kiran *et al.,* 2013). Turmeric composed of 3.5% minerals, 5.1% fat, 6.3% protein, 69.4% carbohydrates and 13.1% moisture, while the oil of turmeric contains almost 53 percent of Sesquiterrpines, 25% of zingiberene, 1% of cineol, 0.5% of borneol and 1% of phellandrene. It is also a fair source of fat soluble vitamin Retinol which is about 91mg and 100 g of its providing 310 kcal. Curcumin 71.5%, bisdemethoxycurcumin 9.1% and desmethoxycurcumin 19.4% are basically the non-evaporative fraction of turmeric (Chattopadhyay *et al*., 2004).

Its powder is used for the cure of cough, anorexia, liver disorders, abdominal disorders and diabetic wounds (Aggarwal et al., 2006). It has cure for inflammation, fungal infection, mutagenesis, carcinogenesis, hepatotoxicity, sterility, fibrosis, cholesterol, diabetes, ulcer, hypertension, viral diseases and coagulation problem. Now days it is used against Alzheimer's, Rheumatoid arthritis, bowel disease, Multiple sclerosis, HIV and Cataract (Jeevangi, 2013). Curcumin is physically a crystalline orange yellowish powder which is water insoluble. Remaining bioactive compounds includes which have lower oxygen scavenging potential including beta sitosterol, beta carotene, p-coumaric acid, terpinene, turmerin, camphene, turmeronola, vanillic acid, turmeronol-b, campsterol and syringic acid (Naz *et al*., 2010).

Diabetes is one of the most common non-communicable pathological condition in the world (Zimmet *et al*., 2001) about a population of 38.2 lac is suffering from the disease and by the year of 2035 this figure will become 55.2 lac (International Diabetes Federation (IDF 2014). DM is a group of different ailments whose indication is high glucose level. Diabetes in human can be allocated into insulin-dependent diabetes, which is distinguished by juvenile onset, due to absolute insufficiency of insulin and by ability to formation of ketone bodies in unavailability of insulin treatment; and other type is noninsulin

dependent diabetes, distinguished by mature onset, by difference in basic insulin levels, have a persistent relationship with obesity, and have a reduce active insulin response to intravenous glucose. Insulin is a hormone which is responsible for maintaining normal blood glucose level by promoting glucose consumption by processes like glycolysis and glycogenesis while it inhibits the glucose-producing processes like gluconeogenesis and glycogenolysis (Koolma *et al*., 2005). Beta cell of the pancreas synthesized and secreted insulin to maintain glucose level of blood within a narrow range (Henquin, 2000). Glucose is one of the most important stimulating agents for insulin production and secretion. An increase in glucose concentration of blood activate the beta cells of pancreas to take-up the C6H12O6, which phenomenon is carried out by an insulin dependent transporter protein named as (GLUT-2) (Thorens, 2001). Turmeric & curcumin reduced sugar level of blood in alloxen induced diabetes in rats (Arun *et al.,* 2002). Curcumin makes better the resistance of insulin in diabetic patients because it increases the making of many genes which are responsible for insulin response due to the presence of peroxisome proliferator activated receptor gamma and its ligand binding function. It also triggers the (Cl^-) entry into the cell for the regulation of insulin secretion when glucose level of plasma is high (Best *et al*., 2007).

Diabetes Mellitus is basically a set of metabolic disorders affecting up to 23 million people in the USA and about 250 million people globally, is distinguished by high blood glucose due to abnormality in insulin release and insulin action, or both. Curcumin longa L. has been generally used for a long time in local medicine for cure of several inflammatory conditions and other diseases. Turmeric yellow pigmented part has the medicinal properties.

2. Materials And Methods

2.1. Procurement of raw material

The randomized controlled trail was conducted in Fruits and Vegetables Processing laboratory, National Institute of Food science and Technology, Faculty of Food, Nutrition and home sciences, University of Agriculture Faisalabad. For analysis turmeric powder will be purchased from local market.

2.2. Preparation of sample

The rhizome of turmeric firstly washed and after washing kept it into the hot air cabinet dryer for drying at the temperature of 60^{o}C for duration of 8 to 10 hours. Then by the help of grinder this dried rhizome of turmeric grounded to obtain a fine powder of turmeric. This turmeric powder for further analysis was stored at normal temperature.

2.3 Preparation of turmeric extract

Turmeric extracts were prepared by using three solvents; methanol, ethanol and acetone following the protocol of Bagchi *et al.* (2012).

T1		50%
T2	Methanol	70%
T3		90%
T4		50%
T5	Ethanol	70%
T6		90%
T7		50%
T8	Acetone	70%
T9		90%

2.4 Extract Analysis:

2.4.1. Total Phenolic Content:

TPC in Turmeric extract will be measured using Folin-Ciocalteu method as mentioned by Himesh *et al*. (2003).

2.5. Efficacy Trial

For efficacy trial experimental animals were divided into 5 groups. study I will be a negative control comprised of Normal rats, study II will have comprised on sterptozocotin induced diabetic rats while study III, IV and V comprised of diabetic rats plus intraperitoneally injected methanolic, ethanolic and acetonic extract respectively. Feed and drink intake and body weight was also measured throughout study experimental period and blood samples was collected at the end of the study period to assess the following parameter.

*Study I	*Study II	Study III	Study IV	Study V
Negative control	Positive control	(Diabetic+Methanolic extract)	(Diabetic+Ethanolic extract)	Diabetic+Acetonic extract)
5	5	5	5	5

*Study 1= Normal rats
*Study 2= Diabetic rats

2.6. Glucose and insulin level

In each study, collected serum was evaluated for glucose concentration by GOD-PAP method as described by Kim *et al*. (2011). Whereas insulin level was measured by following the method of Ahn *et al*. (2011).

2.7. Glutathione assay

Glutathione peroxidase was assayed by the method of Arun et al. (2002). Whereas, reduced glutathione (GSH) was determined by the method of Arun et al. (2002).

2.8. Thiobarbituric acid reactive substances (TBARS). The concentration of thiobarbituric acid reactive substances (TBARS) was determined in the tissue by the method of Donnan (1950).

3. Result and discussion

For the cure of many diseases, a nutrient which contains bioactive compounds attaining a lot of importance. Spices are the real source of these phytochemical compounds. Real research which is related to the health benefits of turmeric is not available in Pakistan. The main purpose of this study is to find out the beneficial effect of turmeric for the cure of different diseases specially focused on Diabetes. First of all nutrient composition of turmeric was assessed followed by the conventional extraction of turmeric by different fluids. Then the best extraction solvent was selected and given to the rats and then evaluated the effect of these extracts on the glucose level of rats. After that the results were statistically interpreted to check the significance of study.

3.1. Conventional antioxidant capability for different solvents

There is a significant effect of percentage of the solvent while a highly significant effect of type of the solvent on extract of turmeric oxidation preventing profile. While, the relation or the interaction between percentage and type of the solvent was seen non-significant. Total phenolic content (TPC) means related to three solvents at their different concentrations. The trending values for different which have been observed shows that the ethanolic extract of turmeric at the concentration of 70% gives the best result for TPC and its total phenolic content was 1006.56 ± 12.12 mg GAE/100mg followed by methanolic extract at the concentration of 70% gives the TPC value 599.62 ±6.56 GAE/100mg and from acetone the best extraction percentage was also 70 % and its TPC value was 594.94 ± 1.54 (Table 1).

Table 1 Total phenolic content of Turmeric (GAE /100g)

Solvent	Treatments			Mean
	T1 (50%)	T2 (70%)	T3 (90%)	
Methanol	**596.20 ± 6.05**	**599.62 ±6.56**	**594.72 ± 5.51**	**596.85b±6.04**
Ethanol	**1002.8 ± 10.20**	**1006.56 ± 12.12**	**998.71± 11.63**	**1002.68a±11.32**
Acetone	**592.49 ± 5.48**	**594.94 ± 4.54**	**591.84 ± 6.76**	**592.93b±5.59**
Mean	**730.49ab±7.24**	**733.31a±7.74**	**728.26b±7.96**	

3.2. Antioxidant potential

Antioxidant potential is basically the oxidation preventing capability of any compound. It's the ability of the compound to prevent the body from harmful effects of oxidation by scavenging the free radicles or by stopping the production of free radicles.

3.2.1. DPPH Assay

A picture can be drawn from the statistical analysis that there is a highly significant effect of percentage of the solvent on the total DPPH and also have the highly significant effect of type of the solvent on the DPPH. While, it has been observed that the interaction between percentage and type of the solvent was non-significant.

Nine types of treatments were used to check the DPPH value. DPPH value was checked by three concentrations of methanol, 50, 70, 90 % ethanol 50. 70 and 90 % and after that with acetone using the same three percentages. The trending values for different solvents which have been observed shows that the ethanolic extract of turmeric at the concentration of 70% gives the best result for DPPH and its maximum value was 66.58 ±2.89% followed by methanolic extract at the concentration of 70% gives the DPPH value 63.06a±2.26% and from acetone the best extraction percentage was also 70 % and its DPPH value was 42.96 ±2.16 (Table 3). The result of the present study was supported by the study of Sultan *et al.,* (2014) he finds out the percentage of DPPH content in ethanolic extract he reported the DPH content 52.36%.

Table 2. Table of Variance for DPPH value of Turmeric

Source	df	SS	MS	F
Percentage	2	892.22	446.11	79.65**
Solvant	2	3303.25	1651.62	294.90**
Percentage*Solvant	4	52.71	13.18	2.35^{NS}
Error	18	100.81	5.60	
Total	26	4348		

Table 3. Treatment means of DPPH (%)

Solvents	Treatments			Mean
	T1 (50%)	T2 (70%)	T3 (90%)	
Methanol	51.80±2.74	63.06±2.26	54.49±2.03	**56.45b±**
Ethanol	50.73±2.5	66.58 ±2.89	61.03±2.15	**59.45[a]±**
Acetone	27.97±2.20	42.96±2.16	32.96±2.24	**34.93[c]±**
Mean	**43.50[c]±**	**57.53[a]±**	**49.50[a]±**	

3.3. Efficacy trial

Animal study was conducted to check the effect of curcumin on sterptozotocin induced diabetic rats. Animals were handover to the animal supervisor to keep the rats in controlled conditions for the estimation of the different parameters of the body. Efficacy study was consisting of five different groups or studies firsts study S1 comprised of control group, while study 2 (S2) comprised of the rats in which diabetes is induced by streptozotocin, study 3 (S3) comprised of the rats who are receiving second best selected 70% methanolic extract, while study 4 comprised of those rats who are receiving best selected 70% ethanolic extract while rats of fifth group (S5) are receiving 70% extract of acetone. In start the glucose value of the rats was taken for the comparison of the glucose at last day of the study. There drink, and feed intake were taken on the weekly basis. The performance of blood test and the related hepatic and kidney functioning tests were performed at the last day of study. Then the parameters which are investigated are statistically interpreted.

3.3.1. Drink intake

Drink intake values of the rats has been taken on every week. During a period of four weeks drink intake has been noticed and the mean values of four not shows a big difference only a slight difference has been seen in all the groups. The minimum water intake was seen in S1 group which consist of normal rats their water intake at first week was 25.76±1.04 while water intake at 4th week was decreased to 22.89±3.22 in normal rats. While maximum water intake has been seen in S_2 group this group consists of rats that are diabetic and did not receiving any extract. The water intake at 1st week of trail was 25.88±.51 while at the 4th week the water intake increases up to 29.26±.78. The significant effect on the water intake has been seen in rats of group S_4 who are diabetic and receiving the ethanolic extract their intake decrease from the rats of diabetic rats who are not receiving any extract their drink intake at first week was 20.68±2.56 while at 4th week the drink intake was up to 25.43±3.06. Maximum drink intake has been seen with acetonic extract in group 5 at 4th week drink intake was 28.57±2.27 followed by group 3 27.40±1.22. So, these least significant changes show that turmeric based extract was tolerable, and it can be used with its potential health benefits.

Table 4: Mean square table for the effect of different treatments on the water intake of rats

Weeks	S1	S2	S3	S4	S5	Means
1st week	25.76±1.04	25.88±.51	26.26±3.01	20.68±2.56	26.77±2.36	25.07
2nd week	23.09±2.72	26.78±1	24.66±2.73	23. 63±2.53	28.23±2.01	25.28
3rd week	25.06±2.82	28.91±0.29	28.77±1.35	28.14±1.86	26.65±1.2	27.51
4th week	22.89±3.22	29.26±.78	27.40±1.22	25.43±3.06	28.57±2.27	26.71
Means	24.20	27.71	26.77	24.47	27.55	

3.3.2. Intake of Food

Food intake values of the rats had been taken on every week. During a period of four weeks feed intake has been noticed and the mean values of four weeks show a significant difference in all the groups. The maximum food intake was seen in S1 group which consist of normal rats their water intake at first week was 26.43±1.05 while feed intake at 4th week was increased to 28.88±1.50 in normal rats. While minimum water intake has been seen in S2 group this group consists of rats that are diabetic and did not receiving any extract. The water intake at 1st week of trail was 23.45±2.14 while at the 4th week the water intake increases with a slight change up to 23.76±2.65. The significant effect on the water intake has been seen in rats of group S4 who are diabetic and receiving the ethanolic extract their intake increase from the rats of diabetic rats who are not receiving any extract their feed intake at first week was 22.55±2.50 while at 4th week the feed intake was up to 28.66±1.49. Minimum feed intake had been seen with acetonic extract in group 5 at 4th week drink intake was followed by group 26.10±1.67 followed by methanolic extract taking group of rats S3 27.12±2.30. So, these least

significant changes show that turmeric based extract was tolerable, and it can be used with its potential health benefits.

Table 5: Mean square table for the effect of different treatments on the feed intake of rats

Weeks	S1	S2	S3	S4	S5	Means
1st week	26.43±1.05	23.45±2.14	23.33±0.35	22.55±2.50	25.51±1.01	24.25
2nd week	28.24±0.65	26.78±2.03	25.52±1.95	26.76±1.80	26.19±0.48	25.61
3rd week	28.69±0.99	24.82±2.84	25.19±1.77	29.37±2.51	26.65±1.20.45	27.25
4th week	28.88±1.50	23.76±2.65	27.12±2.30	28.66±1.49	26.10±1.67	26.90
Means	28.06	23.34	25.29	26.84	26.49	

3.3.3 Glucose level:

A picture can be drawn from the statistical analysis that there was a highly significant effect of days on the glucose level of rats and have the highly significant effect of different studies on the glucose level. While, it has been observed that the interaction between days and type of study was non-significant.

The study was conducted to check the effect of methanolic extract, ethanolic extract and acetonic extract of turmeric on the glucose level of the body. The glucose level of rat's changes with respect to other groups significantly. The means of insulin at 0 days for S1, S2, S3, S4 & S5 were 103±10.58, 274.47±2.52, 208±6.62, 191.86±5.45 and 200.73±9.49respectively. While highest effect on the level of glucose has been seen with ethanolic extract 182.57±11.36 followed by methanolic extract 195.43±2.55 effect was seen in those rats who are receiving acetonic extract 214.97±9.44 in control group amount of glucose released was 94d±9.2 while in diabetic rats the amount of insulin recorded was 279.25a±6.

Table 6: Effect of turmeric extract on blood glucose level mg/dl of diabetic rats

Days	S1	S2	S3	S4	S5	Means
0 Days	103±10.58	274.47±2.52	208±6.62	191.86±5.45	200.73±9.49	198.63a±6.93
30 days	85±7.81	284.03±9.5	195.43±2.55	182.57±11.36	214.97±9.44	189.55b±8.13
	94d±9.2	279.25a±6	202.15b±4.5	187.22c±8.5	207.85b±9.5	

3.3.4. Effect of turmeric extract on Insulin

A picture can be drawn from the statistical analysis that there was a highly significant effect of days on the insulin level of rats and have the highly significant effect of different studies on the insulin level. While, it has been observed that the interaction between days and type of study was non-significant.

The study was conducted to check the effect of methanolic extract, ethanolic extract and acetonic extract of turmeric on the insulin level of the body. The insulin level of rat's changes with respect to other groups significantly. The means of insulin at 0 days for S1, S2, S3, S4 & S5 were 13.44±0.63, 8.90±0.24, 9.8±0.49, 11.10±0.67 & 10.28±0 respectively. While highest effect on the level of insulin has been seen with ethanolic extract 10.90±0.34 followed by acetonic extract 10.91 ±0.52 whilst lowest effect was seen in those rats who are receiving methanolic extract 11.30±0.04 in control group amount of insulin released was 13.40 ±0.65while in diabetic rats the amount of insulin recorded was 9.01±0.13.

Table 7: Analysis of Variance for insulin

Source	df	SS	MS	F
Days	1	1.85	1.85	9.33**
Study	4	106.06	26.51	133.72**
Days*Study	4	1.87	0.45	2.35^{NS}
Error	40	7.93	0.198	
Total	49	117.71		

**** Highly Significant ^{NS} Non significant**

Table 8: Effect of turmeric extract on Insulin level of diabetic rats

Days	S1	S2	S3	S4	S5	Means
0 Days	13.44a±0.63	8.90f±0.24	9.87^{bc}±0.49	11.10^{bc}±0.67	10.28^{cd}±0.09	10.72
30 days	13.40a±0.65	9.01ef±0.13	10.90^{bc}±0.34	11.30^{b}±0.04	10.91^{bc}±0.52	11.10
	8.95	13.42	10.39	11.20	10.60	

3.3.5 Glutathione Assay:

From the statistical analysis there is a highly significant effect of groups on the plasma level of the glutathione. So, it shows that the serum level of glutathione was vary from group to group according to different type of extract they are receiving (Table 4.13.1).

The study was conducted to check the effect of methanolic extract, ethanolic extract and acetonic extract of turmeric on the glutathione level of the body. The glutathione level of rats changes with respect to other groups significantly. The means of insulin at 0 days for S1, S2, S3, S4 & S5 were 27.78±1.99, 17.74±4.45, 18.17±3.36, 27.29±4.33and 23.61±3.2 respectively. The maximum effect on 30^{th} day was seen with ethanolic extract the value to glutathione decreased significantly with respect to S2 diabetic rats 29.30±2.57, while minimum effect was seen with acetonic extract 24.55±3.99.

Table 8: Analysis of Variance for Glutathione

Source	Df	SS	MS	F
Days	1	43.80	33.80	9.31**
Study	4	622.01	155.50	500.41**
Days*Study	4	51.28	12.82	2.72^{NS}
Error	20	94.13		
Total	29	811.22		

****Highly Significant** ^{NS} **Non significant**

Table 9: Effect of turmeric extract on Glutathione of diabetic rats

Days	S1	S2	S3	S4	S5	Means
0 Days	27.78±1.99	17.74±4.45	18.17±3.36	27.29±4.33	23.61±3.2	22.92^{b}±3.46
30 days	30.36.13±2.12	15.23±2.4	24.55±2.77	29.30±2.57	26.55±4.79	25.34^{a}±2.93
	29.07^{a}±2.05	16.83^{d}±3.42	21.36^{c}±3.06	28.30^{ab}±3.45	29.07^{bc}±3.99	

3.3.6 TBARS

The study was conducted to check the effect of methanolic extract, ethanolic extract and acetonic extract of turmeric on the TBARS level of the body. The TBARS level of rats change with respect to other groups significantly. The means of insulin at 0 days for S1, S2, S3, S4 & S5 were 3.99±0.63, 7.34±1.24, 5.05±0.49, 4.30±0.67, 5.07±.76respectively. The maximum effect on 30^{th} day was seen with ethanolic extract the value to TBARS decreased significantly with respect to S2 diabetic rats 4.28±0.04, while minimum effect was seen with acetonic extract 4.96±0.59.

Table 10: Effect of turmeric extract on TBARS level in diabetic rats

Days	S1	S2	S3	S4	S5	Means
0 Days	3.99±0.63	7.34±1.24	5.05±0.49	4.30±0.67	5.07±.76	5.155±0.47
30 days	3.84±0.65	5.81±0.87	4.63±0.34	4.28±0.04	4.85±.65	4.68±0.74
	3.91±0.87	6.57±0.76	4.84±0.87	4.29±0.47	4.96±0.59	

4. Conclusion:

Result shows that ethanolic turmeric extract has anti diabetic effects, so turmeric can be used for the treatment of diabetes.

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