Exploring Therapeutic Efficacy of Coriandrum Sativum and Allium Sativum Aggregate in Alloxan-induced Diabetic Mice

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Exploring Therapeutic Efficacy of Coriandrum sativum and Allium sativum Aggregate in Alloxan-Induced Diabetic mice

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ABSTRACT

Diabetes mellitus (DM) is a global health problem with 422 million individuals around the world suffering from diabetes. *Coriandrum sativum* and *Allium sativum* possess numerous bioactive compounds which are of therapeutical significance. This study was undertaken to explore the combined potential of the two herbs in managing diabetes using diabetic albino mice as model. Extracts of both herbs were prepared and were orally administered in 10% Alloxan monohydrate (alloxan) induced diabetic albino mice over 28 days (in four doses with 7 day interval) to determine the optimal therapeutic and lethal doses. Safe dose limit of both extracts was deduced to be below 600 mg/kg (<600 mg/kg). Diabetic mice were given extracts (200 mg/kg and 400 mg/kg) over 56 days at 7 day intervals, and biological parameters were evaluated, at each interval, including Glucose level (mg/dl), HbA1C (%), Hepatic and Renal biomarkers, and Lipid profile. Garlic-coriander combination (at 400 mg/kg) was able to reduce glucose level (i.e.194.5 mg/dl) ($P<0.05$). Also, it showed a worthwhile effect on biological parameters as well as in Hepatic and renal tissues. Histological examination of hepatic and renal tissues indicated a restoration of normal tissue architecture.

INTRODUCTION

Diabetes mellitus is considered as one of the most common causes of death worldwide and is existing as a global health problem[1,2]. As per World Health Organization (WHO), about 422 million individuals around the world are suffering from diabetes and a significant fraction of nationals is from low and middle income countries. About 1.6 million deaths occur due to diabetes each year and a steady rise in the number of cases and prevalence has been observed. Worldwide, diabetes has become the fourth leading cause of non-communicable diseases (NCDs) while the prevalence has risen from 4.7% to 8.5% [3].

Diabetes mellitus exists as type 1 diabetes, which is caused by failure of pancreas to produce sufficient insulin for the body, and type II diabetes which results from the inability of the body to efficiently utilize insulin leading to enhanced blood glucose concentration in body; complications can result including hypertension, kidney disorder, eye damage, nerve damage, damage to blood vessels, lower lib amputation, heart damage, stroke and blindness [3,4].

At present, for controlling glucose level, the modern medicines recommended for diabetes are classified into six categories (i.e. biguanides (metformin), alpha-glucosidase inhibitors, meglitinides (glinides), sulfonylureas, thiazolidinediones (glitazones), and DPP-4 inhibitors) and two types of injections [4]. However, several side and adverse effects of such medications are known including gastrointestinal disorder, nausea, diarrhea, compromised renal function, de-compensated heart failure, liver disease, weight gain, cardiovascular risks and increased risk of heart attack [5].

People worldwide are more concerned and conscious about the side effect and the cost effectiveness of drugs and, as a complementary or alternative approach, herbal therapies (being used by about one-third of the patients for managing diabetes) are getting increasing attention, which are comparatively less exploited for their medicinal qualities [6]. Herbal drugs having anti diabetic, anti-hyperglycemic as well as insulin sensitizing activities are increasingly sought after by diabetic patients and healthcare professionals [7]. Hence, there is need to explore therapeutic effects of medicinal plants against diabetes and associated conditions [8].

Coriandrum sativum (coriander) possesses a variety of bioactive molecules which can have anticancer, antioxidant, neuroprotective, analgesic, anxiolytic, anticonvulsant, hypolipidemic, hypoglycemic, hypotensive, antimicrobial, and anti-inflammatory activities [9]. *Allium sativum* (garlic) is also reported to have many therapeutic benefits due to presence of sulphur-containing compounds (e.g. S-allylcysteine sulphoxide) and other effective molecules that can have anti-thrombotic, antioxidant, antibiotic, hypocholesterolaemic, hypoglycaemic and hypotensive activities [10]

In the present research, effect of garlic and coriander dose combinations were administered in alloxan induced diabetic mice and estimation of positive effects on metabolism including blood Glucose level, Cholesterol, HbA1C, Cholesterol, Hepatic and Renal biomarkers as well as histological examination of Hepatic and Renal tissues.

MATERIALS AND METHODS

Collection And Maintenance Of Experimental Animal

Male albino mice (100) were purchased from the University of Health Sciences (UHS) Lahore. Experimental mice were kept housed in the animal house of the University College Of Pharmacy, University of The Punjab under standard animal house conditions (23±2°C; and 45-50% humidity, 12/12 hour dark and light cycle) and provided with pelleted diet and water ad libitum. The animals were restrained in steel cages and were maintained as per the guidelines of the committee for the purpose of control and supervision of experiments on animals and the experimental protocol was approved by the animal ethical committee of the University College of Pharmacy, University of the Punjab, Lahore[11].

Collection And Authentication Of The Plant Material

Medicinal plant specimens were collected from their natural habitat i.e. southern region of Punjab (Pattoki / Okara) Pakistan and were authenticated by a plant taxonomist of Botany Department of University of the Punjab[12] and authentication numbers were allocated as LAH#261120 for *Allium sativum* and LAH #251120 for *Coriandrum sativum*.

Drying And Pulverization Of Plants

The whole plant of *Coriandrum sativum* and *Allium sativum* was separated, pulverized and dried under shade for 10 days, and stored in sealable zipper plastic bag till further use[13].

Plant Extract Preparation And Percentage (%) Yield

Plant extracts were prepared using six different solvents i.e. Methanol, Ethanol, Petroleum Ether, Ethyl Acetate, Chloroform And Water. Total 1 kg medicinal herb was procured and powdered. Powder was macerated by soaking 500g in 1000ml of solvent in a conical flask of 1000 ml, covered with cork and Aluminium foil, and left for 7 days with mixing, followed by filtration after 7 days. Residues remaining on filter paper were again soaked in solvent for 3 days followed by rotary evaporation at 40°C and stored in tarred glass vial at temperature of 4-8°C.

Percentage yield of each extract was calculated by following formula and was used as reference medicinal material for further experiments[14-16]:

Percentage Yield (%) = Weight of dry extract (w/w)/ Weight of powder of plant (w/w) x 100

Organization Of Animals In Sets For Experimental Design

For the in vivo study, total 60(+/-15) healthy albino mice weighing 22-26 grams were selected at random from the animal house. The grouping was done as shown in Table 1. Mice in all treatment groups were treated orally by gavage needle with 0.3 ml of coriander extract and 0.3ml of garlic extract at doses mentioned in Table 1 according to the body weight for 56 days with 7 day interval. The extract and dilution exhibiting the best result in continuity to the goals and objective of the designed study were opted for further analysis[17,18]. Pioglitazone was used as standard control where 3mg of drug was administered.

Experimental groups	No. of Mice(s)	Treatments for 56 days
Group –I Normal Control	6	Green fodder / Water
Group –II Diseased Control	6	Alloxan 130mg /kg Once
Standard Control –III	6	Pioglitazone 3mg/kg
Group –IV	6	Garlic 200mg /kg
Group –V	6	Garlic 400mg /kg
Group –VI	6	Coriander 200mg /kg
Group –VII	6	Coriander 400mg /kg
Group – VIII	6	Coriander 200mg /kg, Garlic 200mg / kg
Group – IX	6	Coriander 400mg /kg, Garlic 400mg / kg
Group – X	6	Coriander 400mg /kg, Garlic 400mg / kg, Pioglitazone 3mg/kg

Table 1: Organization of animals in sets for experimental design

Disease Induction in Experimental Animals

Alloxan, one of the common diabetogenic agent mainly used in diabetes studies, was administered at different doses i.e. 70mg/kg, 100mg/kg, 120mg/kg, 130mg/kg and 150mg/kg in mice to determine dose for diabetes induction.

Diabetes was established in mice at 130mg/kg dose with maintained blood glucose levels of 200-400mg/dl. Moreover, mortality was not observed at this dose. Mice were kept under observations for seven days to ensure proper induction and stabilization of diabetes[19]. Animals were given green fodder and soya been oil pellets twice a day for the induction of hyperlipidemia.

Optimization of therapeutic dose

For optimization, evaluation was carried out for 28 days with 6 extracts of *A. sativum* & *C. sativum* in different solvents namely methanol, ethanol, petroleum ether, ethyl acetate, chloroform and water were made with five dilutions as 100mg/kg, 200mg/kg, 300mg/kg, 400mg/kg & 500mg/kg.

Four doses were administered in each group with interval of 7 days till 28th day and biological parameters i.e. glucose and cholesterol content (mg/dl) in blood were examined in mice and compared accordingly[20].

Determination of Lethal Dose of *A. sativum* & *C. sativum*

Different extracts of *A. sativum* & *C. sativum* in best responding were made in three

concentrations as 600mg/kg, 800mg/kg, 1000mg/kg dilutions to determine the lethal dose. Four doses were administered in each group with interval of 7 days and the number of alive and dead animals were checked over time till 28th day to determine mortality over time.

Preparation of Blood serum

The blood sample from mice was taken by performing cardiac puncture. The blood sample was collected in the serum get separating tube and allowed to stand at 25°C for 30 min; after that, centrifuged at 3000 x g for about 15 min. The clear serum was collected above the gel while cell debris settled down the gel. The serum was transferred to eppendorf tubes and refrigerated at -80°C for further experimentation[20].

Estimation Of Base Line Values

Baseline values of the blood glucose, serum ALT, AST, albumin, urea, creatinine were determined before the induction of diabetes. The mice were fasted for 24 hours, then intraperitoneal injection of 130mg/kg of 10% Alloxan monohydrate was administered. Mice were given 5% glucose solution orally for 24 hours to prevent the death with hypoglycemia. Diabetes was developed over a period of 3 days. Classical symptoms of diabetes were observed i.e. polyuria, polydipsia and polyphagia within three days of alloxan administration. Mice with blood glucose level in range of 250- 400 were considered as diabetic[20]. Similarly, base line values for HLD, VLD, triglycerides, cholesterol were noted before feeding the fatty diet to animals[21].

Treatment Evaluation

Garlic extract, coriander extract and garlic extract + coriander extract at two different concentrations i.e., 200 mg/kg and 400 mg/kg with eight dose replications (over 56 days with dose administered with 7 day interval) were administered in Alloxan-induced diabetic mice, and at each 7 day interval. Biological parameters including Glucose level (mg/dl), HbA1C (%), Hepatic and Renal biomarkers, and Lipid profile. were estimated for all groups in intervals over 56 days[21].

Histological Evaluations

After completing blood analysis, mice were dissected and small section of heart was cut from all groups. Heart tissues were dried in filter paper and washed with normal saline to remove blood and preserved in 4% formalin. By microtoming, slices of tissues were obtained and observed under light microscope. The slices were fixed by using gelatin on slide and were placed in oven for 10 hours at 58°C, followed by Hematoxylin and Eosin staining[22].

Statistical Analysis

Statistical analysis of data was done by performing two-way Analysis of Variance (ANOVA) with Graph Pad Prism v. 6.0, (Graph Pad Software, San Diego, California, USA). A p-value of ≤ 0.05 was interpreted as result being statistically significant.

RESULTS

Percentage Yields of Herb Extracts

Highest percentage yield was produced with extracts prepared in Ethanol (21.5% for garlic and 23% for coriander) followed by methanol, ethyl acetate, petroleum ether, chloroform and finally water (Table 2). Extract obtained from ethanol were hence used for subsequent experimentation.

		Petroleum Ether	Chloroform	Ethyl Acetate	Methanol	Ethanol	Aqueous
Percentage yield (%)	**Garlic**	14	11	14.5	16.6	21.5	12.5
	Coriander	15	13	15	17	23	18

Table 2: Percentage yield (%) of extracts from A. sativum and C. sativum prepared in different solvents.

Optimization of Therapeutic Dose

On optimization of therapeutic dose of both *A. sativum* and *C. sativum* extracts prepared in various (6) solvents, and five different dilutions i.e. 100mg/kg, 200mg/kg, 300mg/kg, 400mg/kg & 500mg/kg; significant effects in reducing mean values of glucose (mg/dl) and cholesterol level (mg/dl) were observed with the dilutions of 400mg/kg (259.5 mg/dl glucose with garlic extract and 203.25 mg/dl glucose with coriander extract) & 500mg/kg (139.25 mg/dl cholesterol with coriander extract and 132.25 mg/dl cholesterol with coriander extract) concentrations prepared in ethanol (Figure 1a-d). Other extract concentrations prepared in solvents other than ethanol indicated lesser reductions in mean values.

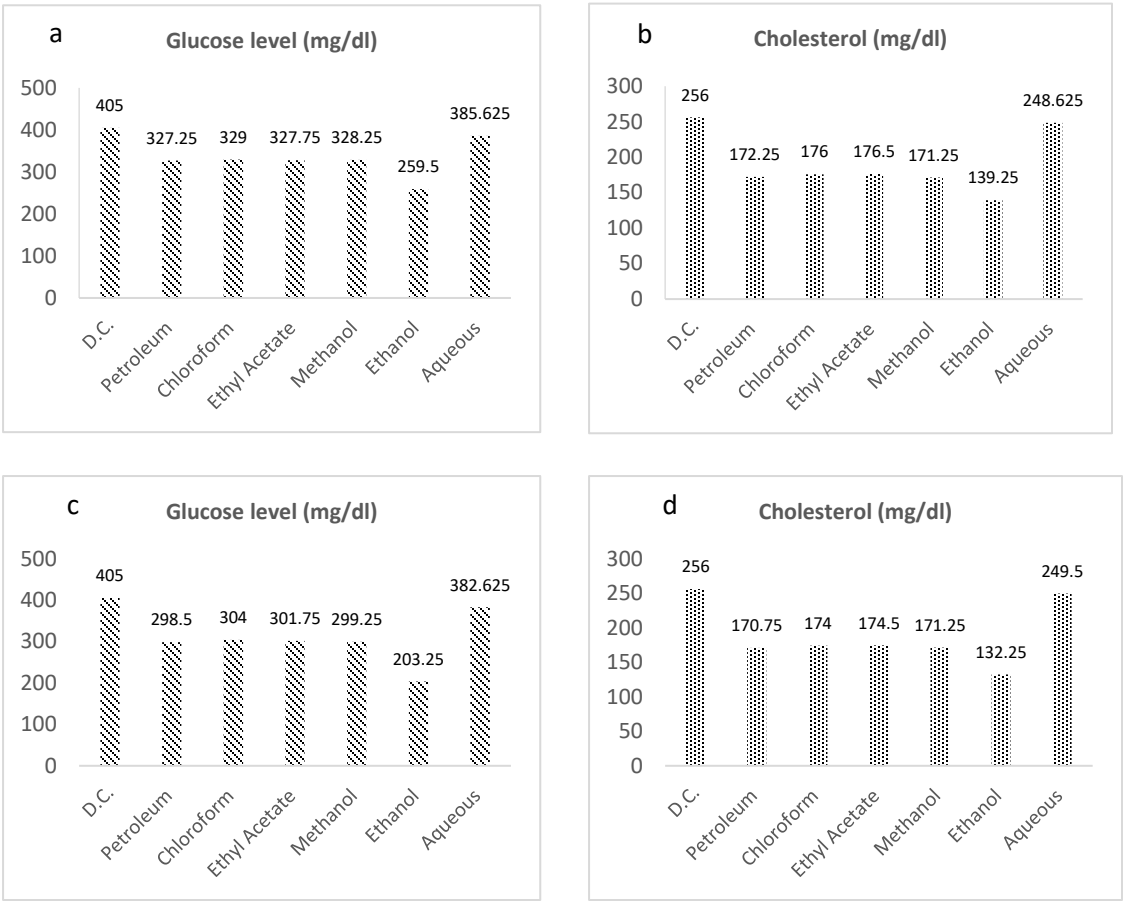

Figure 1a): *Effect of 400mg/kg garlic extract on Glucose level (mg/dl).* ***1b)*** *Effect of 400mg/kg garlic extract on Cholesterol (mg/dl).* ***1c)*** *Effect of 500mg/kg coriander extract on Glucose level (mg/dl).* ***1d)*** *Effect of 500mg/kg coriander extract on Cholesterol (mg/dl).*

Estimation of Mortality by Lethal doses of *A. sativum* & *C. sativum*

Lethal doses of both herbs was evaluated by administering extract at doses 600 mg/kg, 800 mg/kg and 1000 mg/kg for 28 days, and track of mortality of mice was monitored along the time period. From Table 3, it could be seen that the mortality of animals increased with increasing the concentrations of each of the herb type. By 28th day, only one out of six (1/6) animals was alive with 1000 mg/kg garlic extract; while with coriander extract of 800 mg/kg 1/6 animals were alive by Day 21st indicating greater toxicity of coriander extract as compare to garlic extract. Safe dose limit of both extracts was henceforth deduced to be below 600 mg/kg (<600 mg/kg).

Duration	Garlic ethanol extract (mg/kg)			Coriander ethanol extract (mg/kg)		
	600 alive/dead	800 alive/dead	1000 alive/dead	600 alive/dead	800 alive/dead	1000 alive/dead
Day 7th	(6/0)	(5/1)	(5/1)	(6/0)	(5/1)	(5/1)
Day 14th	(4/2)	(4/2)	(4/2)	(4/2)	(4/2)	(3/3)
Day 21st	(4/2)	(3/3)	(2/4)	(3/3)	(2/4)	(1/5)
Day 28th	(3/3)	(2/4)	(1/5)	(2/4)	(1/5)	(1/5)

Table 3*: Determination of Lethal Dose of A. sativum & C. sativum. Mortality was noticed as number of alive mice/ dead mice under experimental conditions.*

Effects on Glucose Level

On the data for estimation of glucose level (mg/dl), the results of two-way Analysis of variance (ANOVA) between three treatments groups Garlic Extract, Coriander Extract and Garlic Extract + Coriander Extract at two different concentrations i.e., 200 mg/kg and 400 mg/kg with eight dose replications (over 56 days with dose administered with 7 day interval).

Results of 200 mg/kg concentration of garlic and coriander extracts showed mild effects in decreasing the blood glucose level while increasing the concentration to 400 mg/kg further decreased glucose concentration; overall garlic extract at both concentrations indicated better control over glucose concentration compared to coriander and disease control (i.e. 298.625 mg/dl with 200 mg/kg garlic extract and 263.5 mg/dl with 400 mg/kg garlic extract while for coriander the values were at 346.625 mg/dl and 307.625 mg/dl, and 405 mg/dl with disease control). A significant decrease in glucose level was noticed when both extracts were used in combination at 200 mg/kg and 400 mg/kg with mean glucose level of 241.875 mg/dl and 194.5 mg/dl, respectively (P=0.000 for all groups) (Figure 2 a-c).

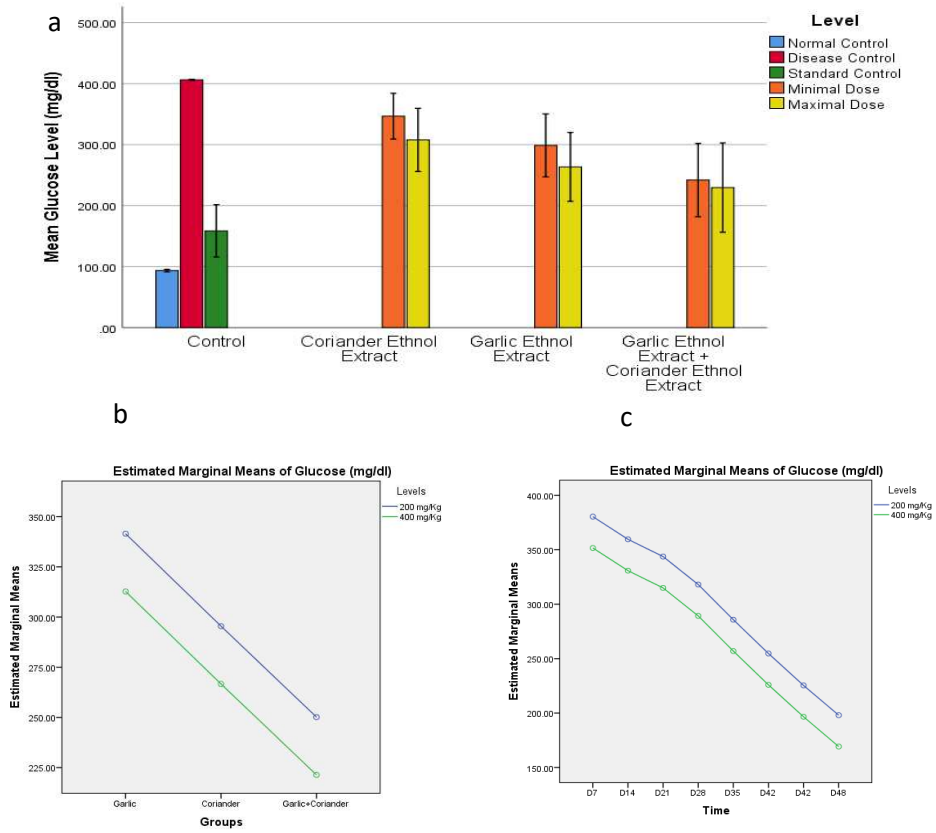

Figure 2a): *Effects of Medicinal herbs on Glucose level (mg/dl) in mice(s). 2**b)** Estimation of doze effectiveness on Glucose level (mg/dl) over two dose levels. 2**c)** Estimation of comparative effectiveness of extracts on Glucose level (mg/dl) over eight equal time intervals.*

Effects on HbA1C (%)

Effect of various doses of garlic and coriander extract on HbA1C (%) of alloxan (130mg/kg) induced diabetic rats was done with garlic and coriander administration alone and in combination (with eight dose replications). The values of HbA1C (%) for garlic, coriander and garlic-coriander combination (400 mg) were quite similar and comparable with the standard control i.e. 8.75% HbA1C, but non-significant as showed by the p-values for coriander (p=0.566) and garlic-coriander combination (p=0.133), while for garlic extract was p=0.000 (Figure 3a-c).

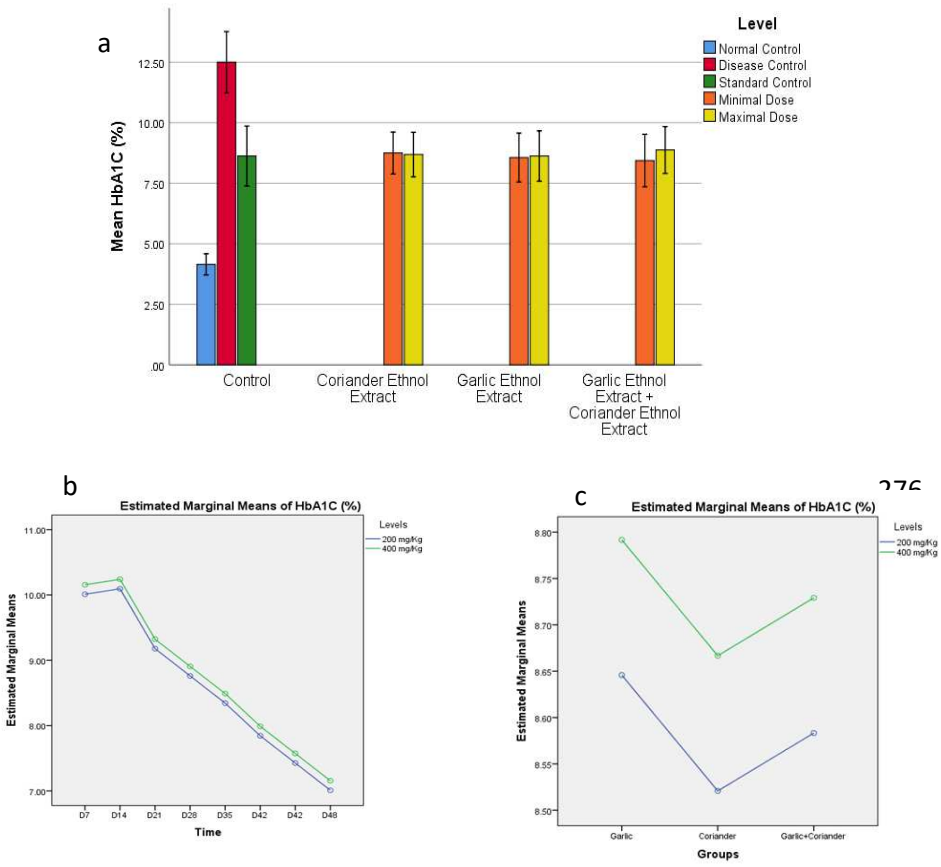

Figure 3 a) *Effects of Medicinal herbs on HbA1c (%) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on HbA1c (%) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on HbA1c (%) over eight equal time intervals.*

Effects on Serum Hepatic Biomarkers

Evaluation of the effect of garlic and coriander extracts on Serum Hepatic Biomarkers indicated effectiveness of both herbs in reducing mean AST level (IU/L) as compared to disease control group (58.45 IU/L) and all test groups of coriander and garlic showed similar effect as with combined effect of both herbs (at 400 mg /Kg) with mean AST level of 43.75 IU/L (p=0.002) while for standard control (PioG) was 40.38 IU/L (Figure 4a-c).

For ALT levels, greatest reduction was shown with combined effect of both herbs (at 400 mg /Kg) with 59.88 IU/L (p=0.002), which matched closely with the value observed with standard control (PioG) was 58.50 IU/L, while the disease control group indicated 103.16 IU/L ALT (Supplementary Figure 1).

ALP (IU/L) levels indicated a similar pattern of decline as that observed with ALT, with means ALP level of 110.50 IU/L (p=0.002) with combined effect of both herbs (at 400 mg /Kg) compared with disease control at 143.56 IU/L, and standard control at 97.25 IU/L (Supplementary Figure 2).

ACP (mM of PNP) levels indicated a similar pattern of decline as that observed with ALT and ALP, with means ACP level of 23.50 mM of PNP (p=0.000) with combined effect of both herbs (at 400 mg /Kg) compared with disease control at 38.56 mM of PNP, and standard control at 20.38 mM of PNP (Supplementary Figure 3).

Mean Bilirubin (mg/dl) values for both herbs were similar indicating both were somewhat effective in reducing the Bilirubin levels and the combined effect of both herbs (at 400 mg /Kg) showed level of 0.87 mg/dl (P=0.016) while the disease control group being at 1.04 mg/dl and standard control at 0.48 mg/dl (Supplementary Figure 4).

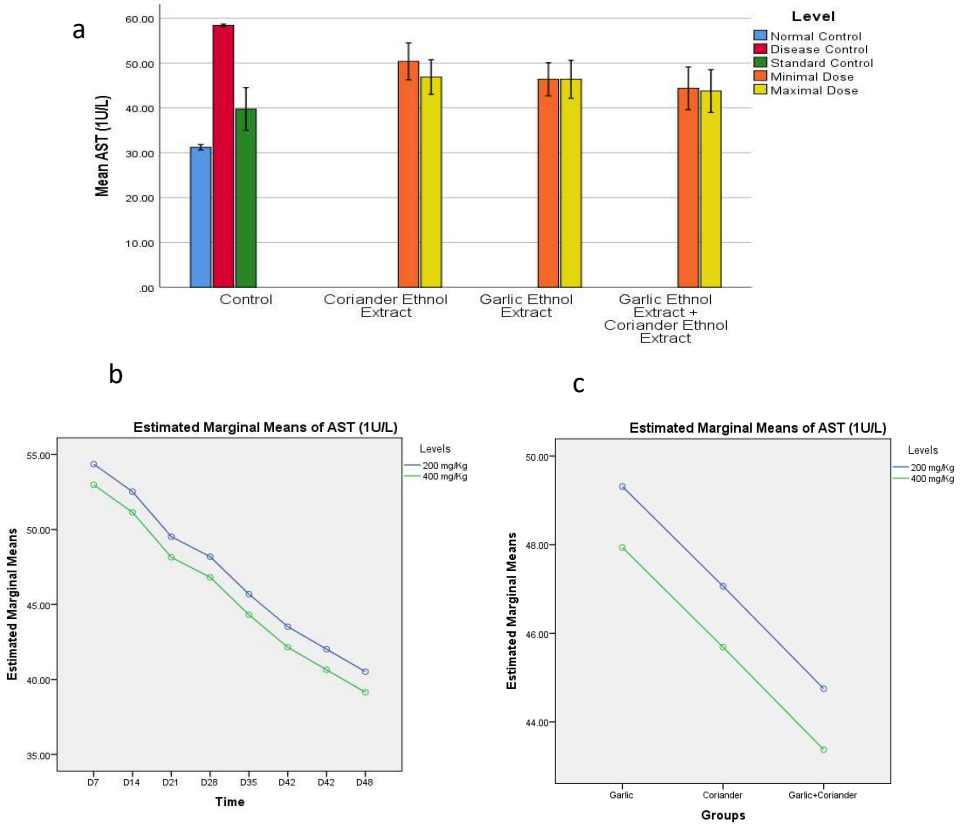

Figure 4a): *Effects of Medicinal herbs on AST (1U/L) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on AST (1U/L) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on AST (1U/L) over eight equal time intervals.*

Effects on Tissue Hepatic Biomarkers

Analysis of tissue Hepatic biomarkers of alloxan (130mg/kg) induced diabetic rats indicated that garlic + coriander (400mg) (administered with dose replications) had mean SOD level 205.63 U/g tissue (p=0.000) while that for disease control group was 178 U/g tissue (i.e. higher values as compared to the disease control group) (Figure 5a-c). Individual effects of herbs (at 200mg and 400

mg) were almost similar, however slightly lesser values of mean SOD (U/g tissue) as compared with the combined effect (at 400mg).

Mean CAT (U/g tissue) and GSH (mg/g tissue) values indicated a similar trend with the dose types and levels of garlic and coriander as observed with mean SOD level (U/g tissue) (Supplementary Figures 5 and 8). Mean CAT (U/g tissue) of garlic-coriander (400mg) indicated mean CAT level of 914.25 U/g tissue (p=0.000) while that for disease control group was 742 U/g tissue.

Mean GSH (mg/g tissue) of garlic-coriander (400mg) indicated level of 3.81 mg/g tissue while that for disease control group was 1.8 mg/g tissue (p=0.000).

The values for mean MDA (nmol/L P*g tissue) (p=0.312) and LPO (nmol of MDA formed/L P*g tissue) (p=0.000) were reduced compared to disease control groups (Supplementary Figures 6 and 7). Mean MDA (nmol/L P*g tissue) of garlic-coriander (400mg) showed value of 42.25 nmol/L P*g tissue (P=0.312) and 52 nmol/L P*g tissue for disease control group; however difference was not statistically significant as per the P value.

Mean LPO (nmol of MDA formed/L P*g tissue) of garlic-coriander (400mg) showed value of 135.75 nmol of MDA formed/L P*g tissue (P=0.000) and 162 nmol of MDA formed/L P*g tissue for disease control group.

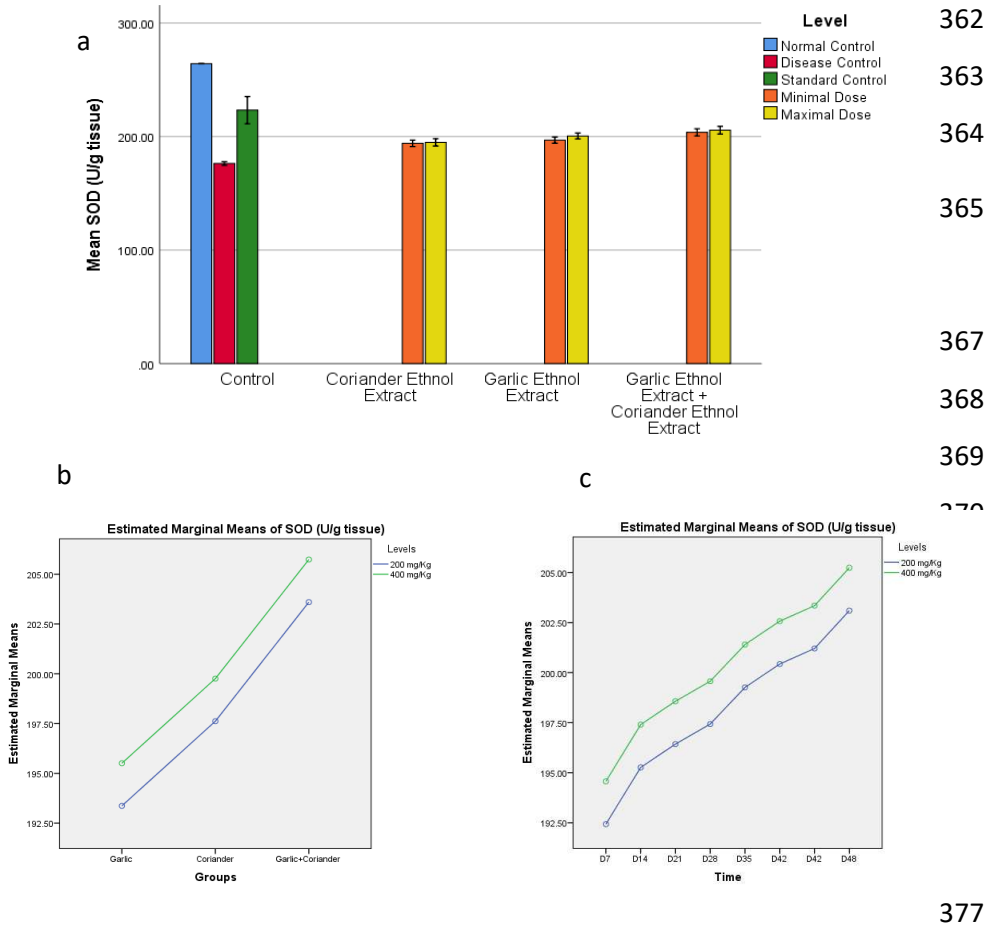

Figure 5a): *Effects of Medicinal herbs on SOD (U/g tissue) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over eight equal time intervals.*

Histological Examination of Hepatic Tissues

Analysis of Hepatic Tissues of drug treated & controlled rats are recorded in Figure 6 a-d. The histological examination showed normal architecture of liver of Normal Control animals (Figure 6a). Normal Control mice showed normal hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes were arranged in trabecules running radiantly from the central vein and were separated by sinusoids containing Kupffer cells. They were regular and contained a large spheroidal nucleus. Also, hepatic cords were radially arranged around the Central vein and Sinusoids. Disease control (Figure 6b) indicated hepatic lobules impaired and cord like arrangements while normal liver cells were lost and the central and portable veins were congested. Liver cell restoration was indicated with garlic and coriander (400mg) (Figure 6c). Lobules arrangement could be seen in que, and central and portal veins were in line and less congested. Similar features were observed with Standard Control as with combination of garlic and coriander (Figure 6d).

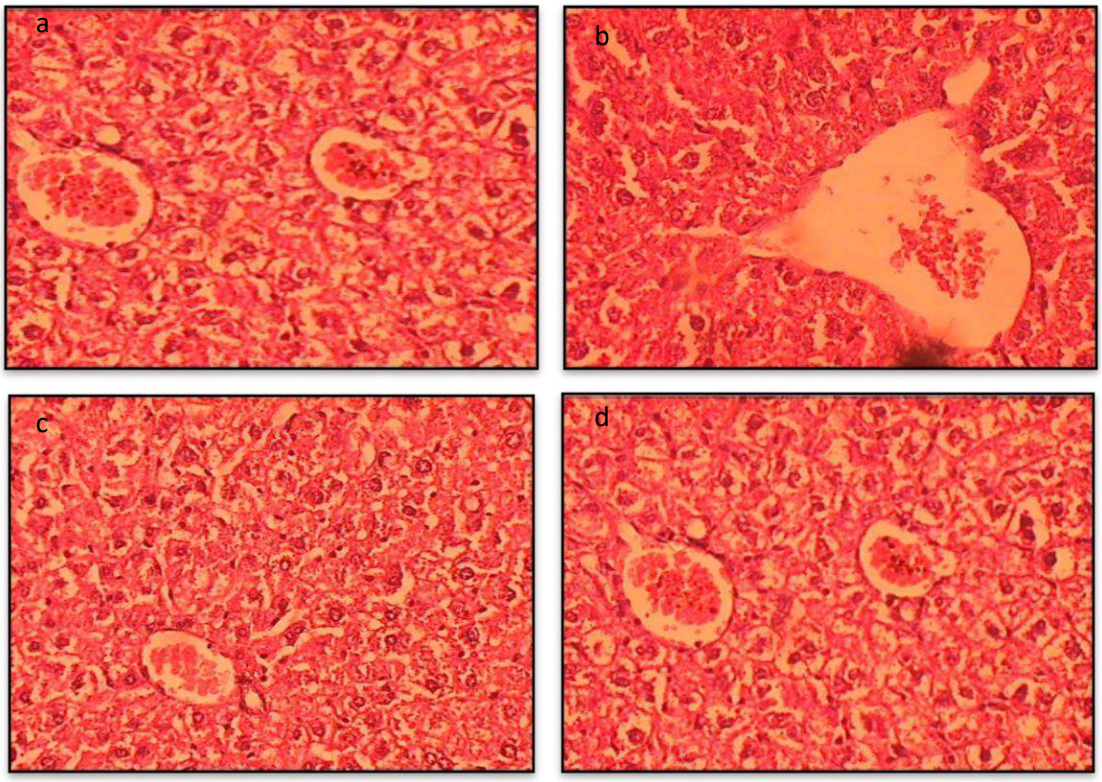

Figure 6: a) *T/S of the Liver of normal control.* ***b)*** *T/S of the Liver of Diseased mice.* ***c)*** *T/S of the Liver of diseased mice treated with 400mg coriander and garlic extract* ***d)*** *T/S of the Liver of Diseased mice treated with Pioglitazone (standard control).*

Effects on Serum Renal Biomarkers

Estimation of mean Urea level (mg/dl) of diabetic mice showed that garlic-coriander extract (400mg) was able to reduce the level to 28.25 mg/dl (P=0.872); but due to statistical insignificant result indicated by P-value, the evidence could not be convincing enough. However, garlic alone (at both concentrations) indicated promising result with 28.38 mg/dl at 200mg (P=0.000) and 29.50 mg/dl at 400 mg (P=0.000), while disease control group had 35 mg/dl urea concentration (Figure 7 a-c).

Result of uric acid concentration (mg/dl) showed garlic-coriander (at 400mg) were able to reduce the level to 3.15 mg/dl (P=0.000) as compared to lower concentrations of garlic and coriander (i.e. 200 mg) as well as disease control (3.80 mg/dl) (Supplementary Figure 9). Creatinine level (mg/dl) level exhibited a similar pattern of decline with 0.96 mg/dl (P= 0.000) with combination of garlic and coriander (400 mg) as compared to disease control 1.17 mg/dl (Supplementary Figure 10).

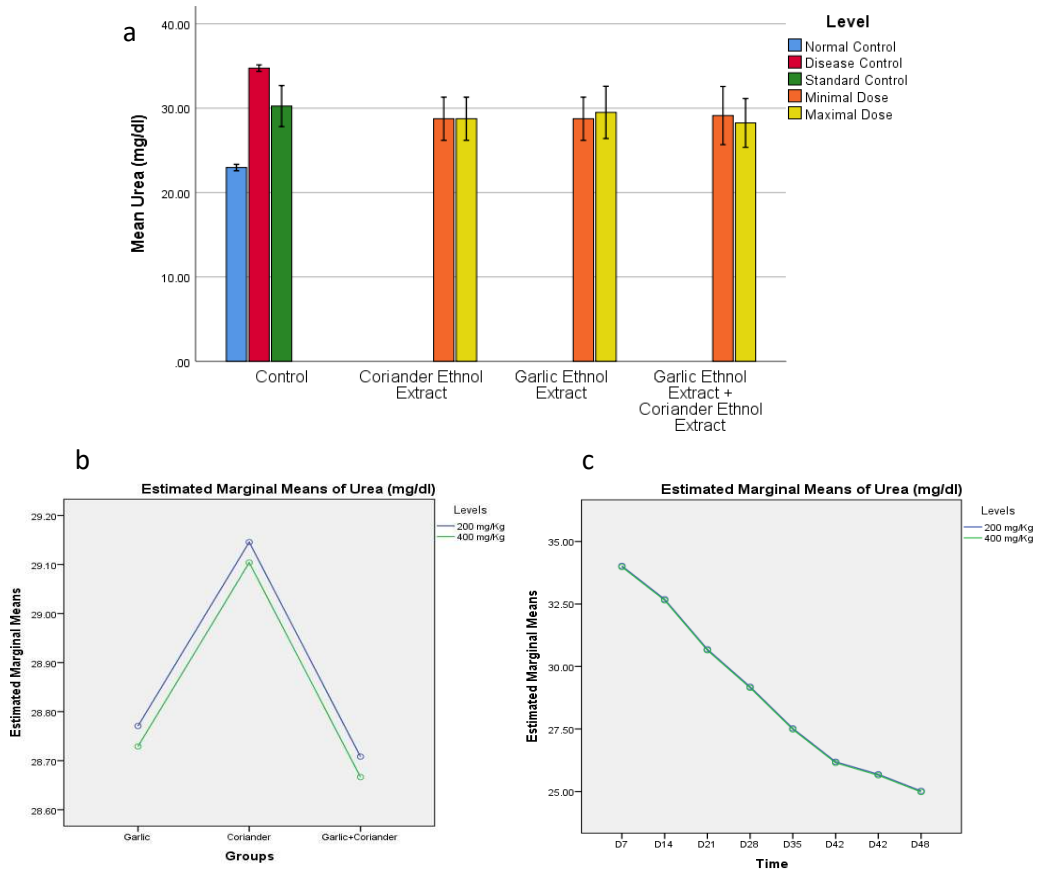

Figure 7a): *Effects of Medicinal herbs on Urea (mg/dl) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on Urea (mg/dl) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on Urea (mg/dl) over eight equal time intervals.*

Effect on Tissue Renal Biomarkers

Estimation of Renal tissue biomarkers showed that mean SOD level (U/g tissue) increased with increasing garlic and coriander dose levels and using combination made a positive effect as compared to the tissues from disease control mice.

Garlic-coriander (400 mg) combination showed a mean SOD level 265.13 U/g tissue (P=0.000) while disease control group SOD level was 231 U/g tissue (Figure 8 a-c).

Similar effects were observed with mean CAT (537.13 U/g tissue for 400 mg combination, P=0.000 and 417 U/g tissue for disease control) and GSH (5.11 mg/g tissue for 400 mg combination, P=0.004, 2.88 mg/g tissue for disease control) (Supplementary Figures 11 and 14).

The values for mean MDA (nmol/L P*g tissue) and LPO (nmol of MDA formed/L P*g tissue) were reduced compared to disease control group. Garlic-coriander extract (400 mg/kg) indicated mean MDA (nmol/L P*g tissue) of 47.88 nmol/L P*g tissue (p=0.064) compared to disease control 52 nmol/L P*g tissue, while the mean LPO (nmol of MDA formed/L P*g tissue) 126.13 (p=0.035)

compared to disease control 139 nmol of MDA formed/L P*g tissue (Supplementary Figures 12 and 13).

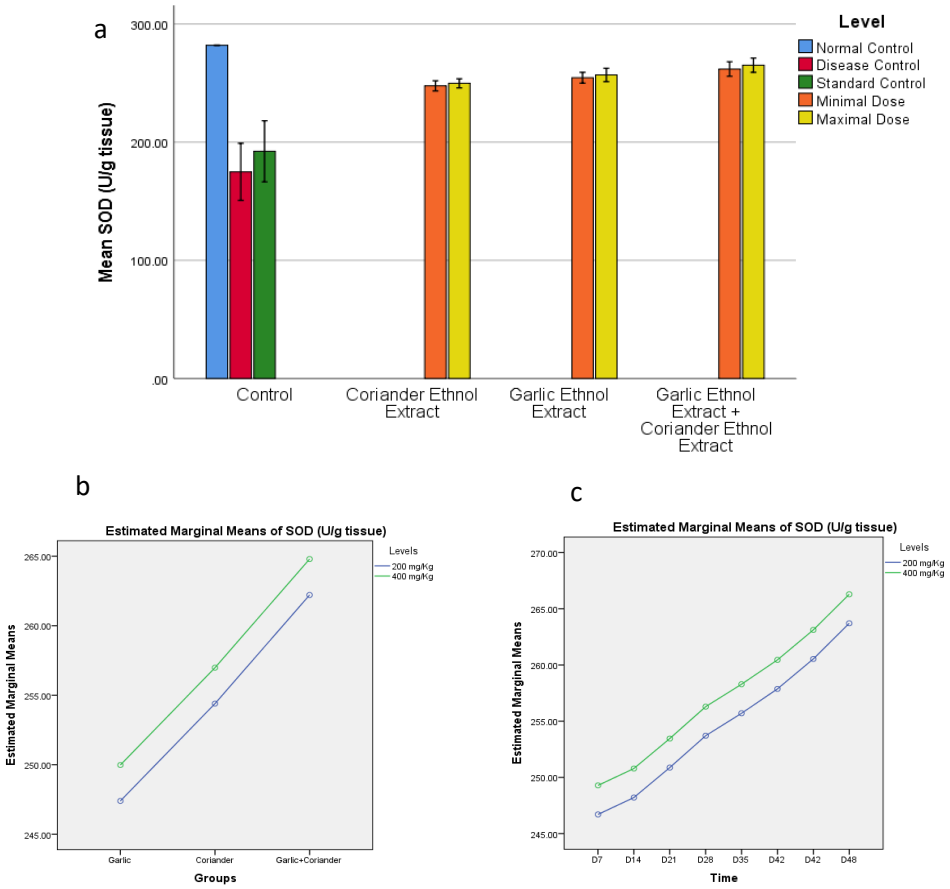

Figure 8a): *Effects of Medicinal herbs on SOD (U/g tissue) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over eight equal time intervals.*

Histological Examination of Renal Tissues

Histological examination of Renal Tissues of drug treated & controlled mice are recorded in Figure 9. Normal renal cortex with well intact renal architecture was observed in normal control mice (Figure 9a). Examination of renal cortex of diseased mice indicated significant dilation of tubules, sloughing of epithelium showing advanced level disintegration of renal tubules as well as shrinkage of glomeruli (Figure 9b). Normal architecture of renal cortex was observed with garlic-coriander extract (400mg), dilation of glomerulus declined, resolving of damage spots, casts were absent while tubules getting compact/ normal (Figure 9c). T/S of renal cortex of mice treated with Pioglitazone (standard control) also indicated improved features (Figure 9d).

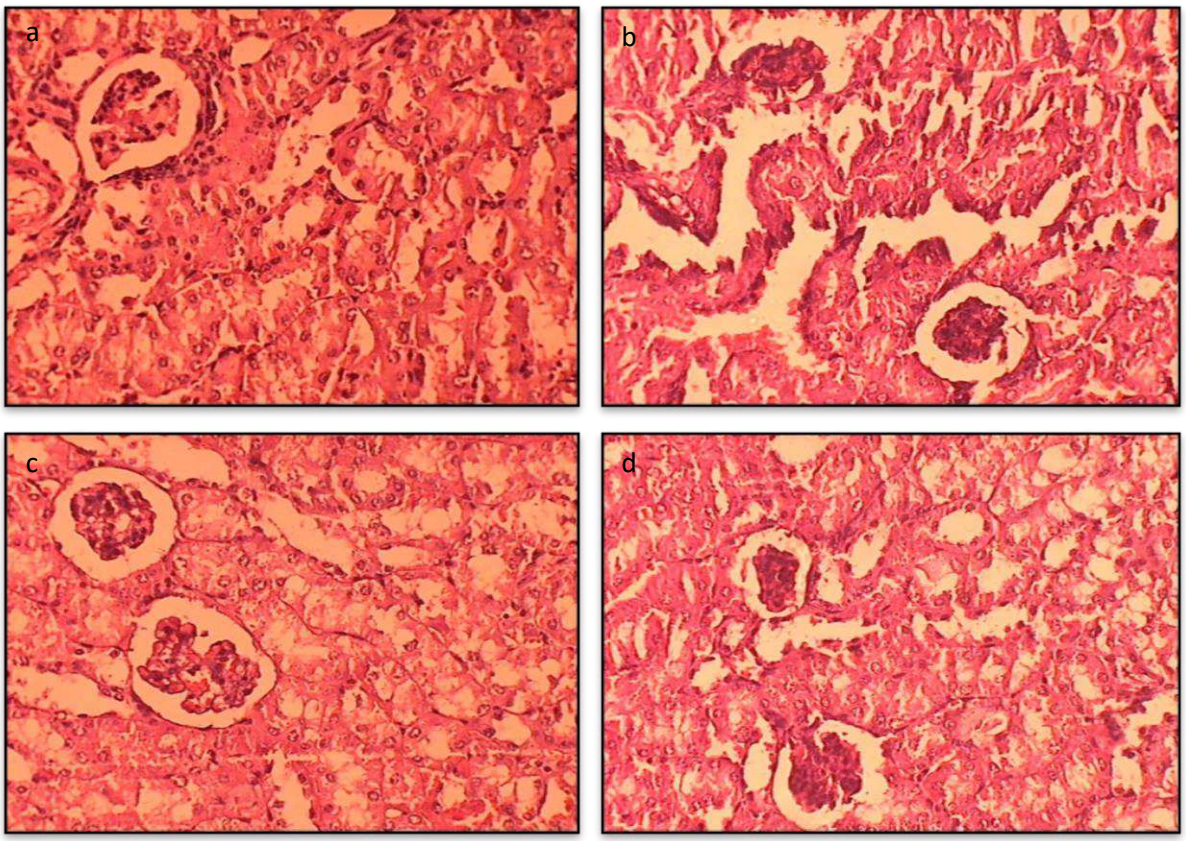

Figure 9: a) *T/S of renal cortex of normal control.* ***b)*** *T/S of renal cortex of diseased mice.* ***c)*** *T/S of renal cortex of mice treated with coriander and garlic extract (400 mg).* ***d)*** *T/S of renal cortex of mice treated with Pioglitazone (standard control).*

Estimation of Lipid profile

Effect of extract on Lipid Profile in alloxan (130mg/kg) induced diabetic rats after eight weeks treatment was evaluated. Significant reduction in mean Cholesterol level (mg/dl) was observed with Garlic and Garlic Coriander combination as compared to disease control mice. Administration of Garlic Coriander extract at 400mg/kg concentration decreased the mean Cholesterol level (mg/dl) to 137.50 mg/dl (P=0.242) in comparison with disease control which was at 256 mg/dl (Figure 10); however, a high P-value making it difficult to reach to a valid conclusion.

The results of Triglycerides (mg/dl) values indicated that Garlic Coriander extract at 400mg/kg concentration decreased the level to 147.50 (P=0.000) as compared to disease control which was at 235 mg/dl (Figure 11) and LDL- cholesterol values (mg/dl) level were reduced to 51.38 (P=0.000) as compared to disease control which was at 93.5 mg/dl (Supplementary Figure 15) while the values for HDL-cholesterol ("good" Cholesterol) were raised to 31mg/dl with Garlic Coriander extract (400mg/kg) as compared to disease control i.e. 15.5 mg/dl (P= 0.001) (Supplementary Figure 16).

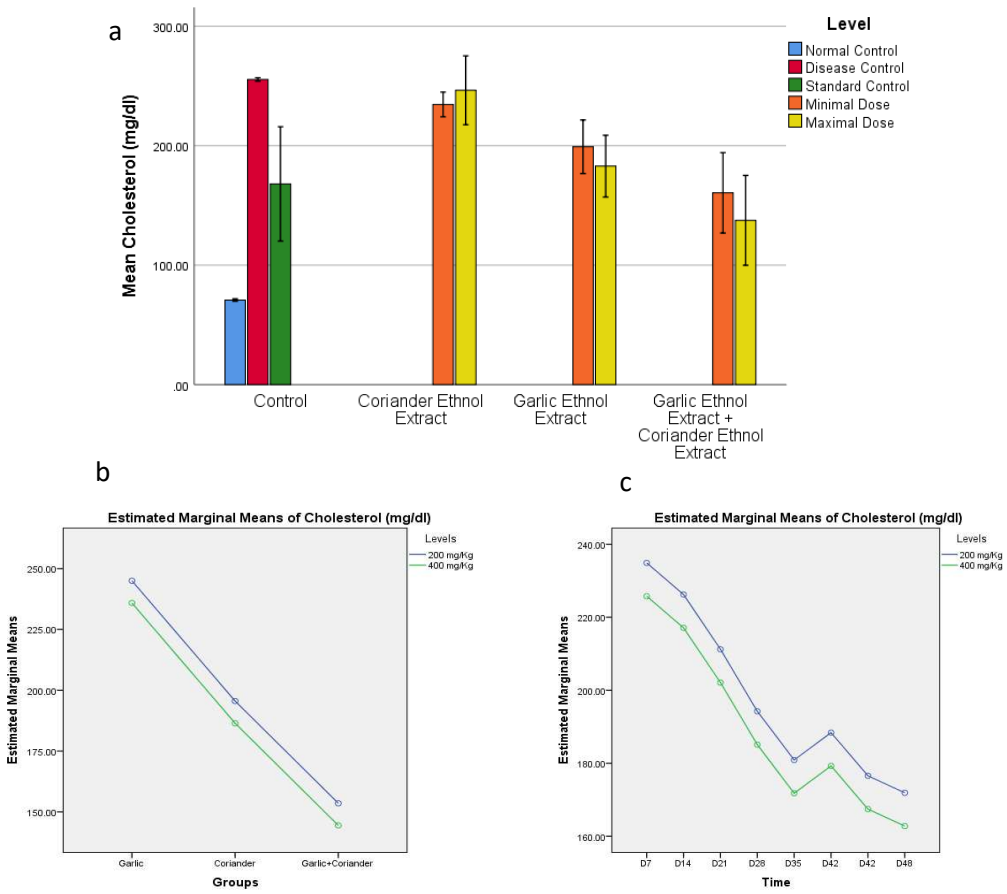

Figure 10a): *Effects of Medicinal herbs on Cholesterol (mg/dl) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on Cholesterol (mg/dl) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on Cholesterol (mg/dl) over eight equal time intervals.*

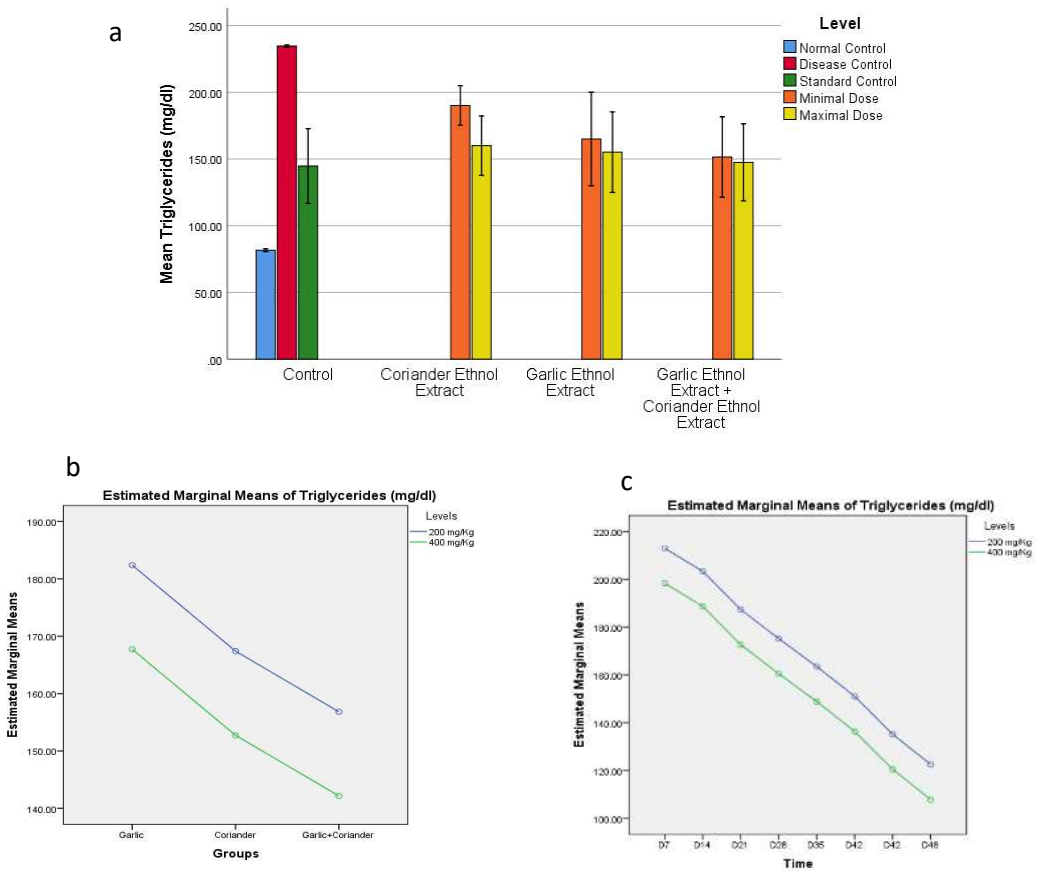

Figure 11a): *Effects of Medicinal herbs on Triglycerides (mg/dl) in diabetic mice(s).* ***b)*** *Estimation of comparative effectiveness of extracts on Triglycerides (mg/dl) over two dose levels.* ***c)*** *Estimation of comparative effectiveness of extracts on Triglycerides (mg/dl) over eight equal time intervals.*

DISCUSSION

Diabetes mellitus (DM) has a strong impact on the quality and length of patients' lives and puts a significant financial burden. The International Diabetes Federation (IDF) diabetes atlas 2017 ranks Pakistan at 10 of 221 countries of the World[23,24]. The most recent diabetes prevalence survey of Pakistan (DPS-PAK) was conducted in 2017 [reported prevalence of diabetes up to 32.9%. Pakistan has 27.4 million cases of diabetes (≥20 years). Data reveals that 415.03% increase in cases of diabetes in Pakistan from 5.32 to 27.4 million[23]. Pakistan, being a third world country, under strong economic burden, and is already allocating a considerable share in dealing with communicable diseases (e.g. (e.g. HIV, Hepatitis B and C), practices of managing a healthy lifestyle by adopting natural remedies for non-communicable disease e.g. diabetes can help to circumscribe the economic burden in health sector.

Use of modern medicines have seeming benefits in diabetic patients, the usefulness is accompanied by various side effects[25], and while some studies have shown anti-diabetic and

cholesterol decreasing properties of *Coriandrum sativum*[26-29] and *Allium sativum*,[10,30-33], studies involving the combined effect of both herbs are scarce[34].

This study was conducted to show the aggregate effect of garlic-coriander combination in adjusting levels of key biochemical indicators in diabetic mice to manageable levels. While the results for certain indicators (i.e. HbA1C, Tissue Hepatic Mean MDA, Serum Renal Urea level and total cholesterol), could not be within limit of statistical significance (i.e. $p \leq 0.05$), other biochemical parameters were well managed by combined dose (i.e. 400 mg/kg) of garlic-coriander and within the limit of statistical significance.

AUTHOR CONTRIBUTIONS

SQ, MB and RA planned the study; SQ performed the analysis, statistical tests and prepared results; BJ helped in analysis and results interpretation; SQ and BJ wrote the manuscript and prepared figures and tables; manuscript was checked by all authors.

ADDITIONAL INFORMATION

The authors declare that there is no conflict of interest.

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Figures

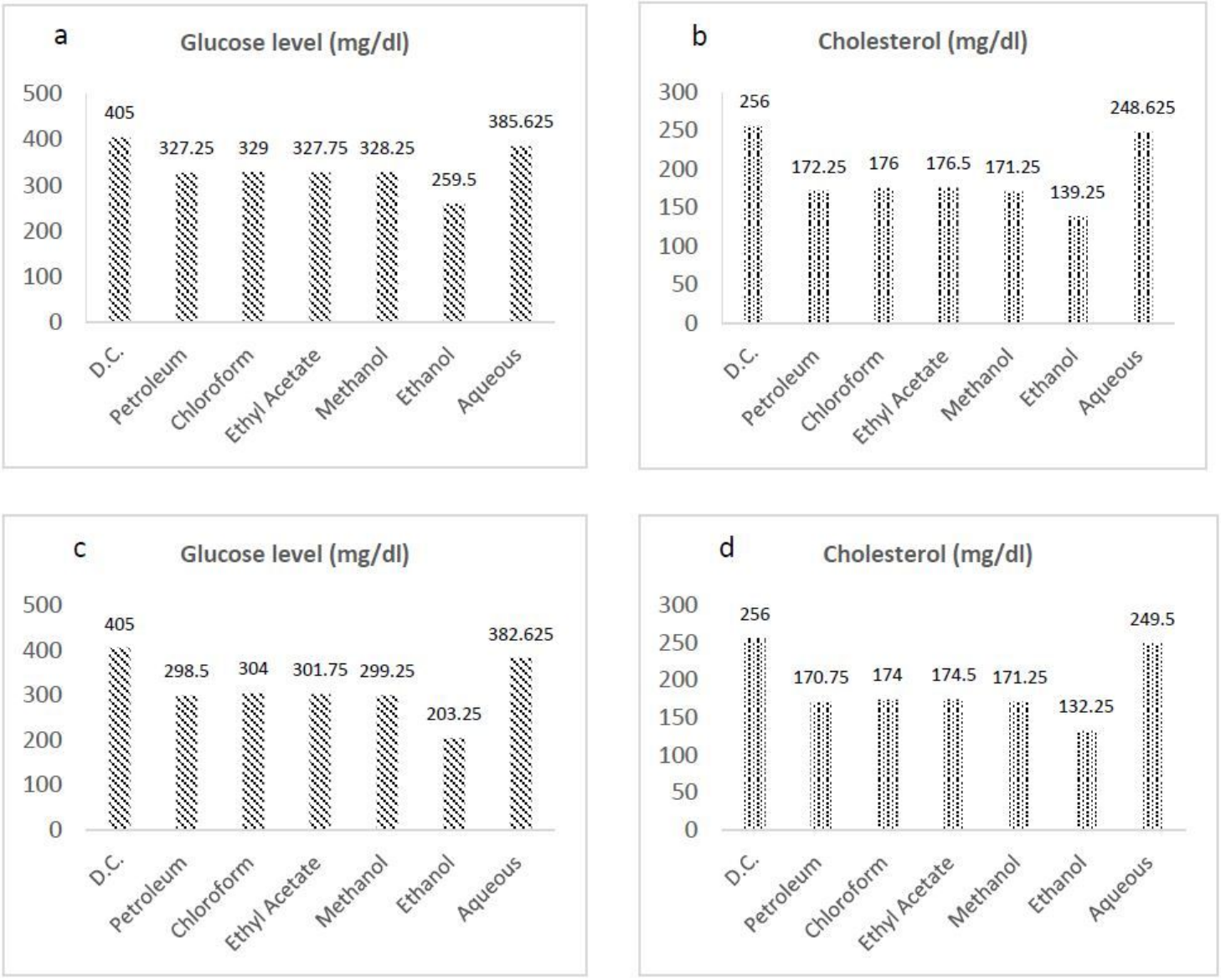

Figure 1

a): Effect of 400mg/kg garlic extract on Glucose level (mg/dl). 1b) Effect of 400mg/kg garlic extract on Cholesterol (mg/dl). 1c) Effect of 500mg/kg coriander extract on Glucose level (mg/dl). 1d) Effect of 500mg/kg coriander extract on Cholesterol (mg/dl).

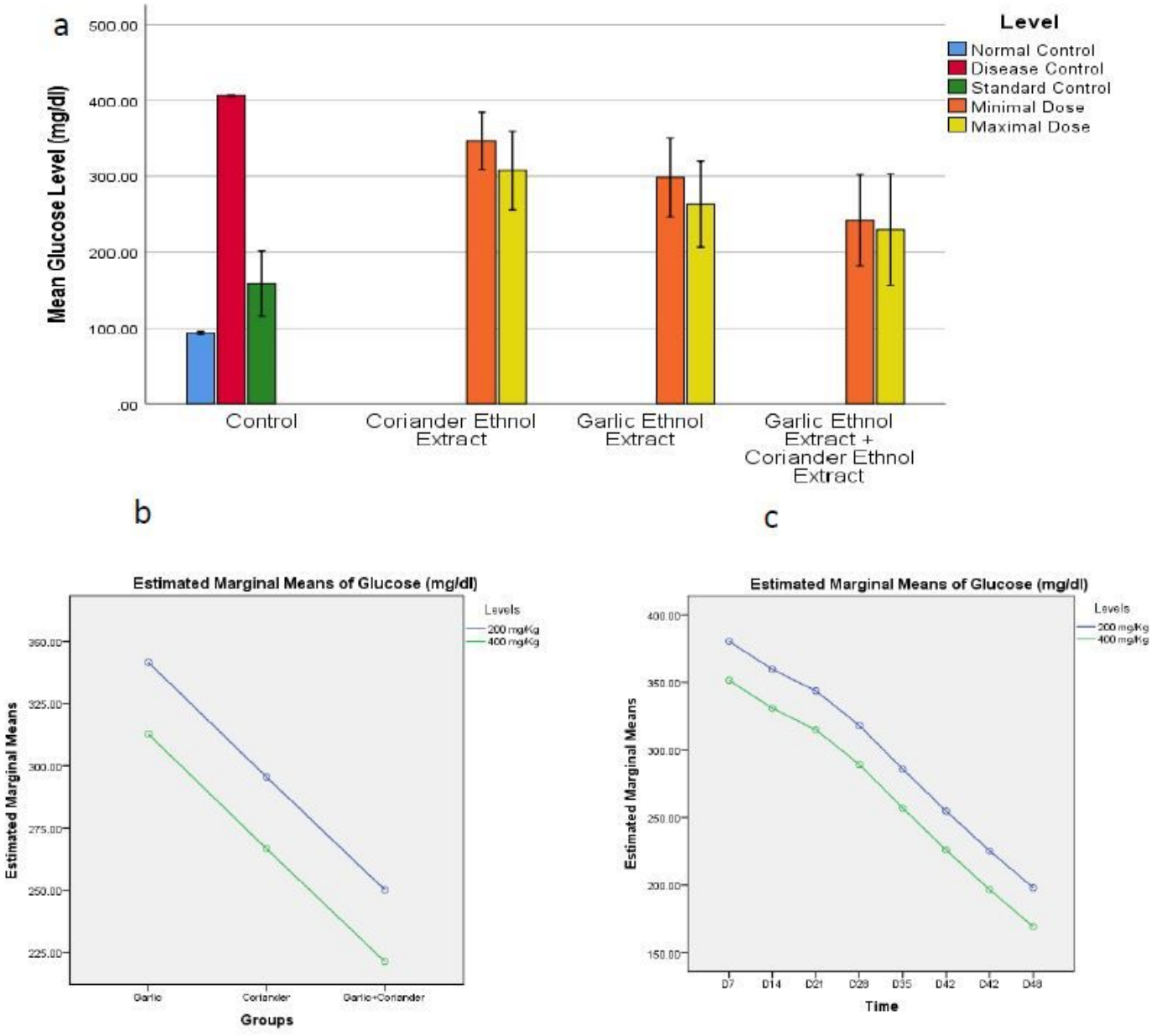

Figure 2

a): Effects of Medicinal herbs on Glucose level (mg/dl) in mice(s). 2b) Estimation of doze effectiveness on Glucose level (mg/dl) over two dose levels. 2c) Estimation of comparative effectiveness of extracts on Glucose level (mg/dl) over eight equal time intervals.

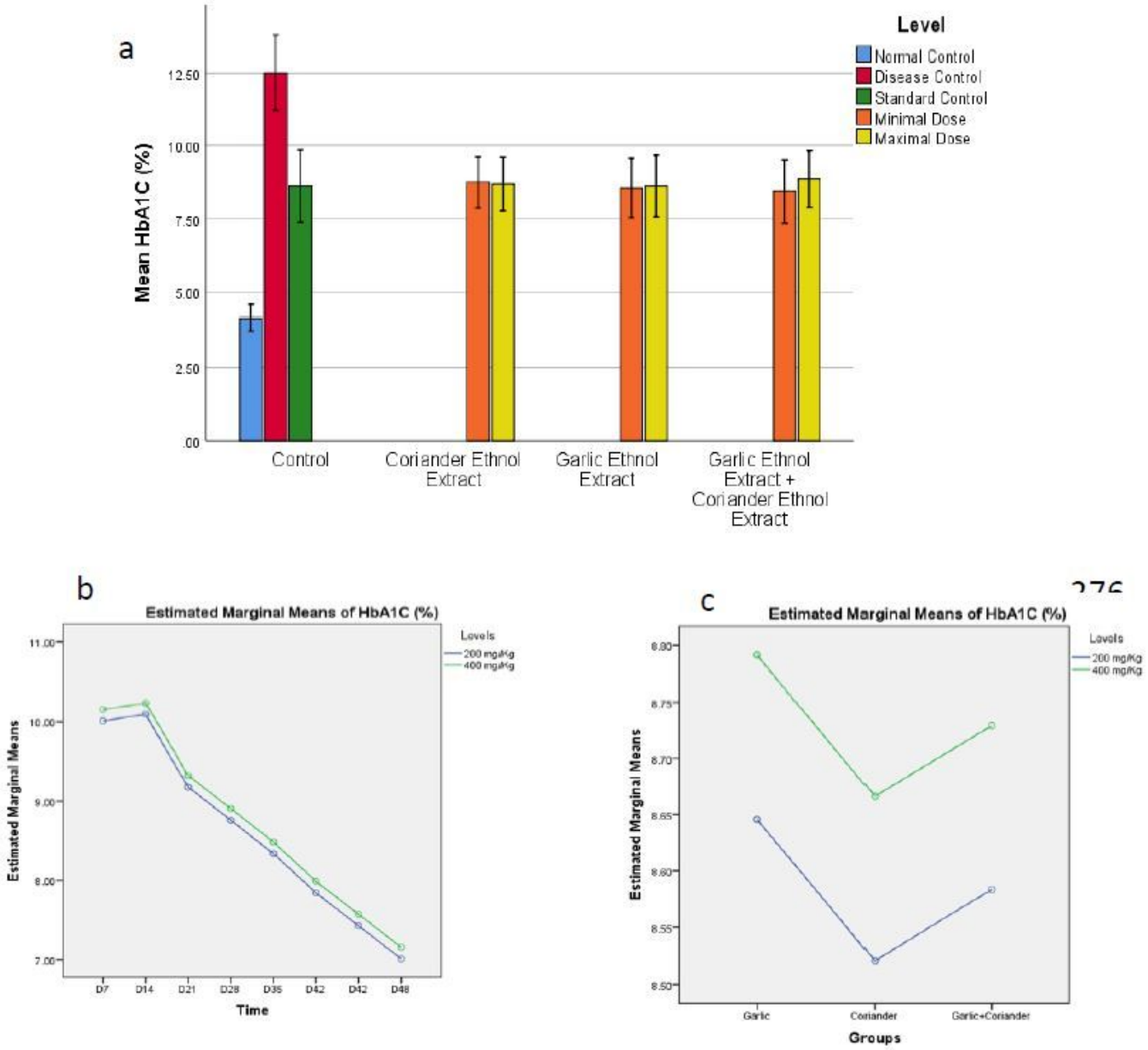

Figure 3

a) Effects of Medicinal herbs on HbA1c (%) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on HbA1c (%) over two dose levels. c) Estimation of comparative effectiveness of extracts on HbA1c (%) over eight equal time intervals.

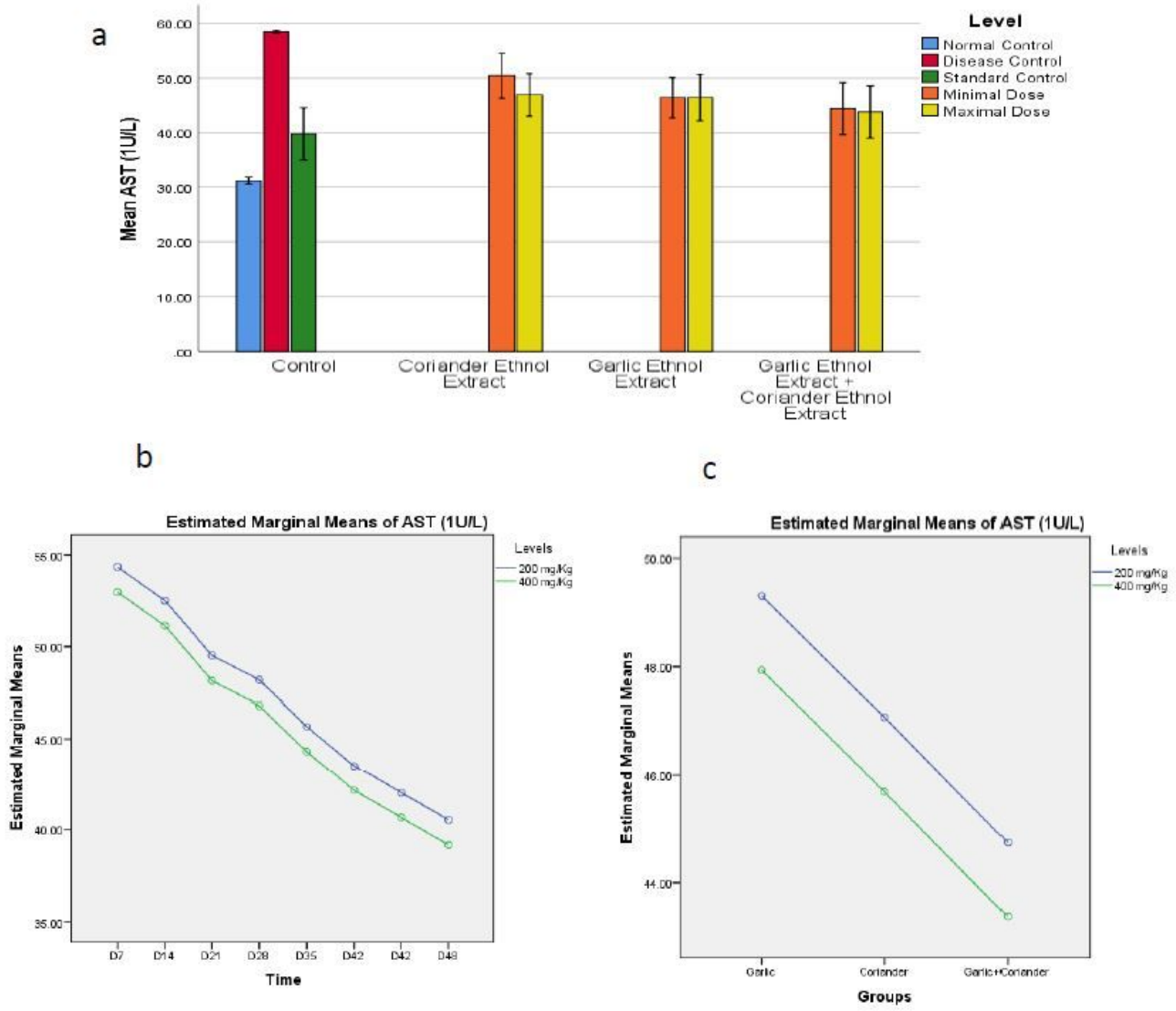

Figure 4

a): Effects of Medicinal herbs on AST (1U/L) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on AST (1U/L) over two dose levels. c) Estimation of comparative effectiveness of extracts on AST (1U/L) over eight equal time intervals.

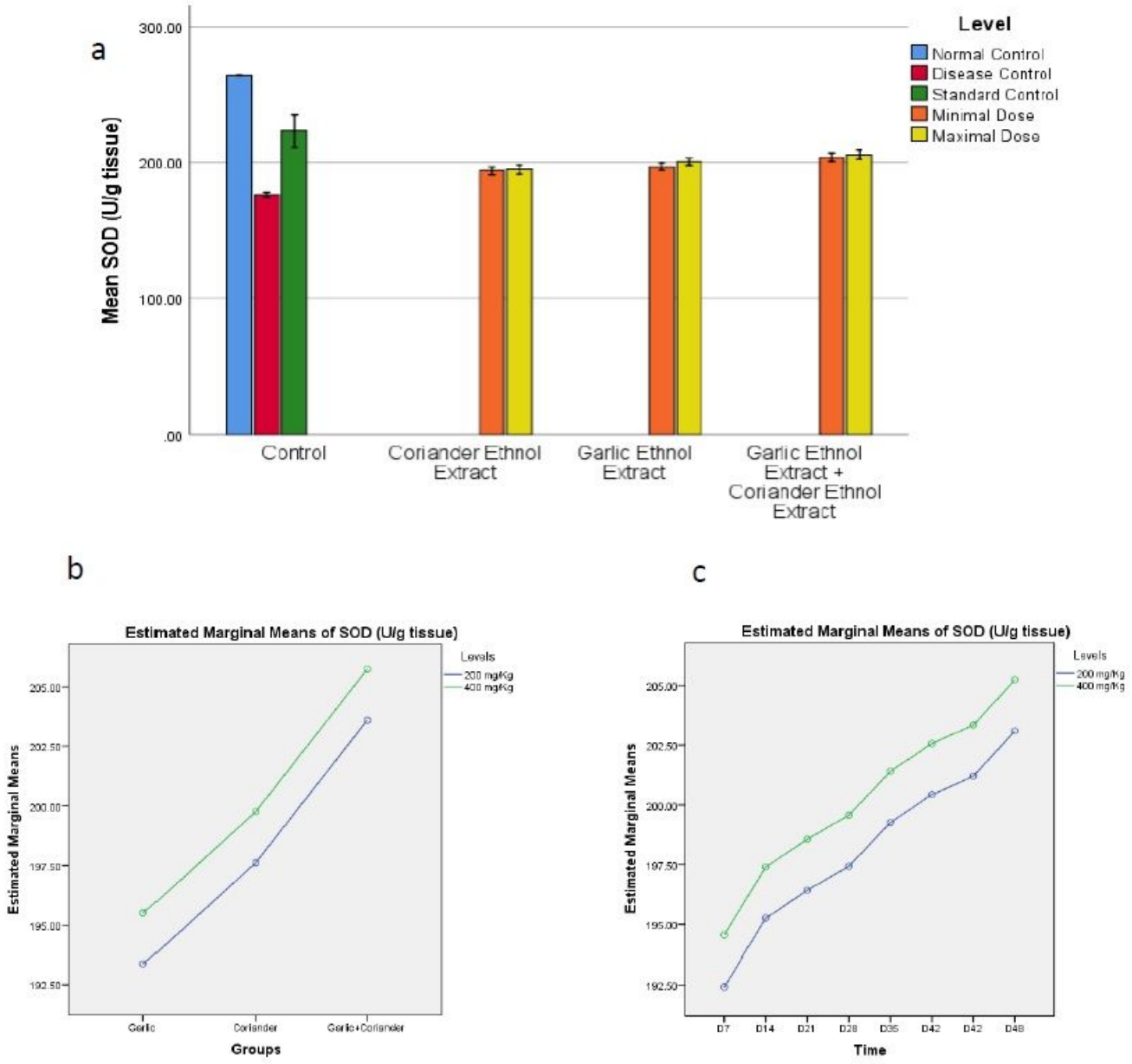

Figure 5

a): Effects of Medicinal herbs on SOD (U/g tissue) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over two dose levels. c) Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over eight equal time intervals.

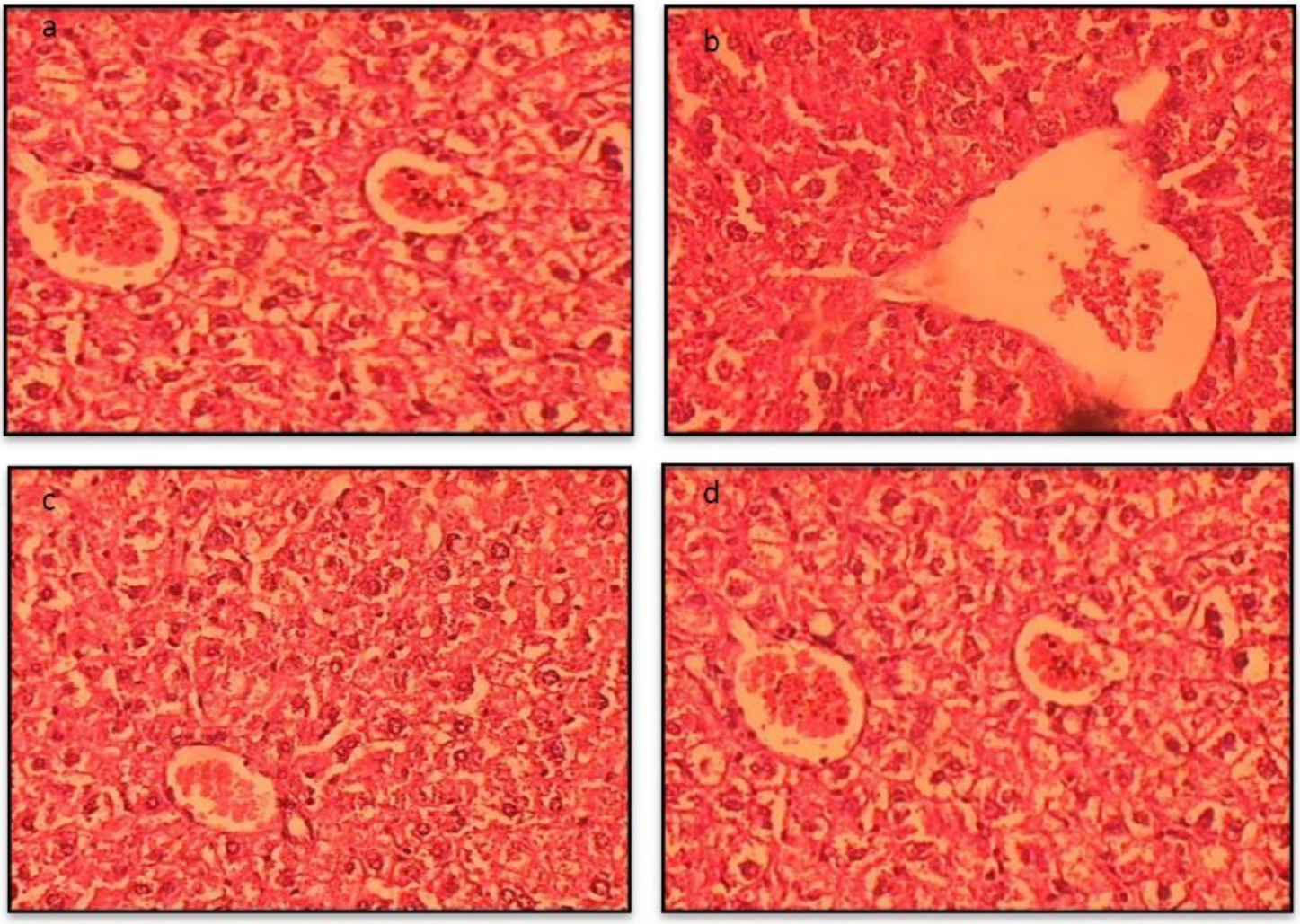

Figure 6

a) T/S of the Liver of normal control. b) T/S of the Liver of Diseased mice. c) T/S of the Liver of diseased mice treated with 400mg coriander and garlic extract d) T/S of the Liver of Diseased mice treated with Pioglitazone (standard control).

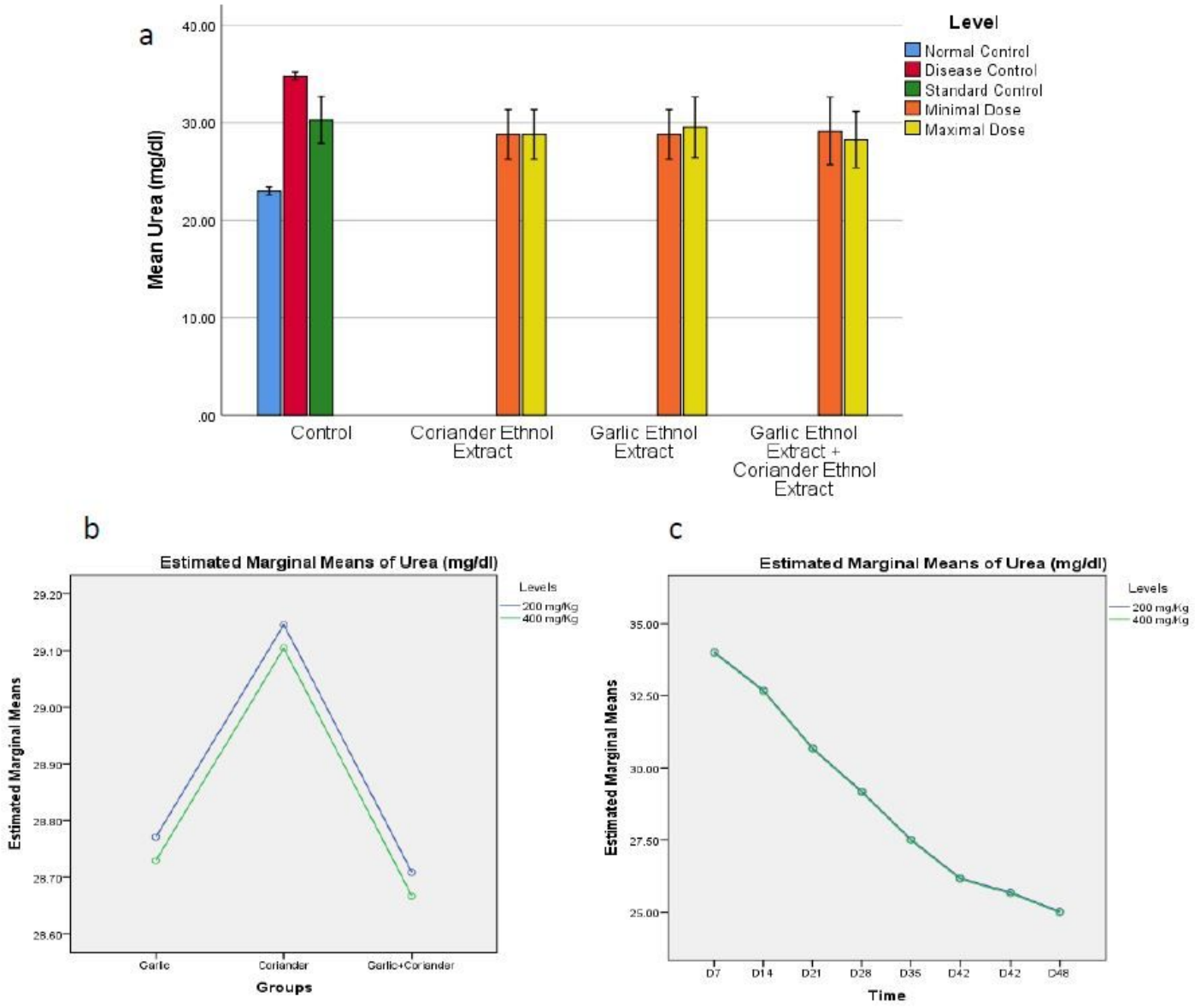

Figure 7

a): Effects of Medicinal herbs on Urea (mg/dl) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on Urea (mg/dl) over two dose levels. c) Estimation of comparative effectiveness of extracts on Urea (mg/dl) over eight equal time intervals.

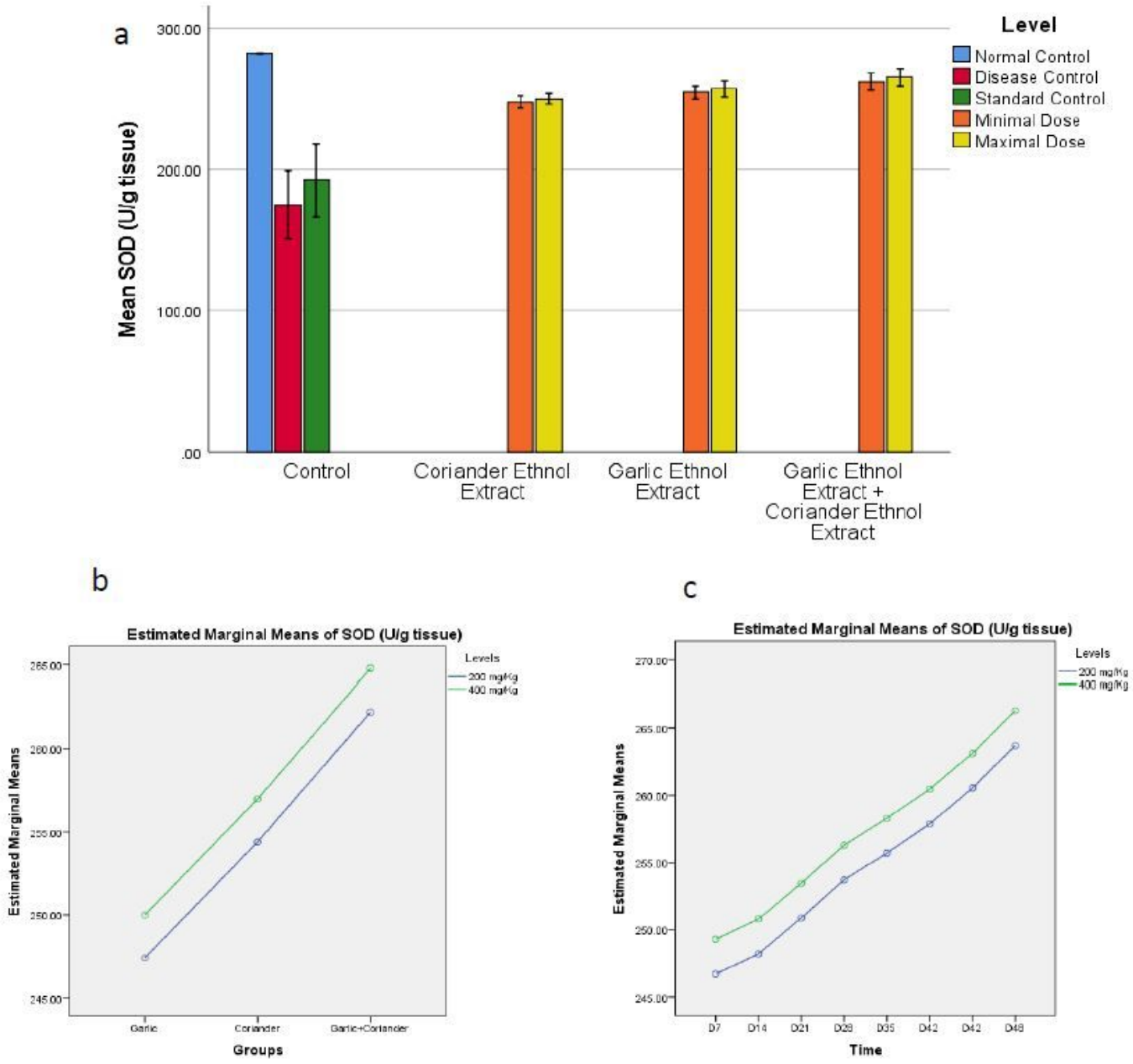

Figure 8

a): Effects of Medicinal herbs on SOD (U/g tissue) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over two dose levels. c) Estimation of comparative effectiveness of extracts on SOD (U/g tissue) over eight equal time intervals.

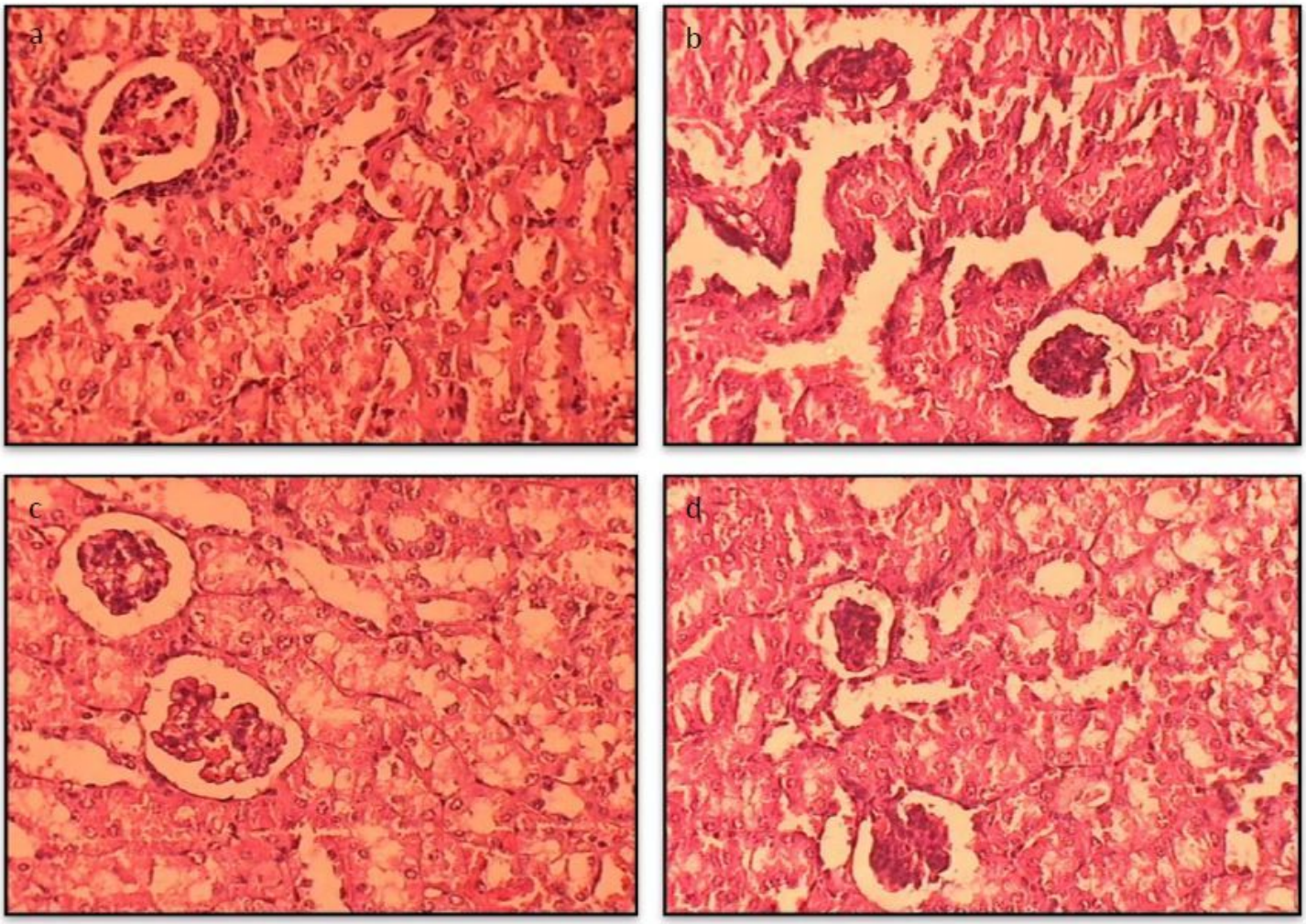

Figure 9

a) T/S of renal cortex of normal control. b) T/S of renal cortex of diseased mice. c) T/S of renal cortex of mice treated with coriander and garlic extract (400 mg). d) T/S of renal cortex of mice treated with Pioglitazone (standard control).

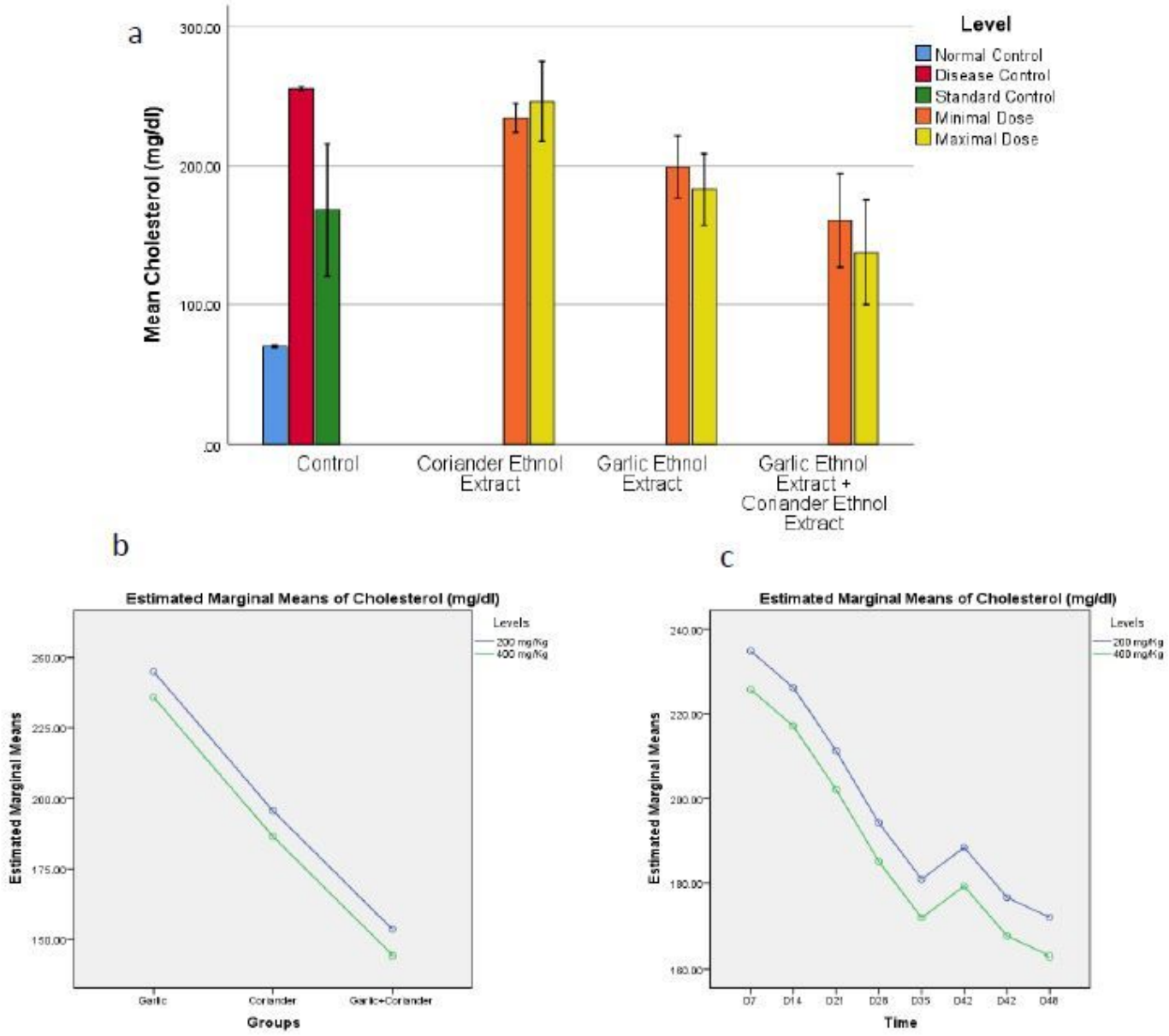

Figure 10

a): Effects of Medicinal herbs on Cholesterol (mg/dl) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on Cholesterol (mg/dl) over two dose levels. c) Estimation of comparative effectiveness of extracts on Cholesterol (mg/dl) over eight equal time intervals.

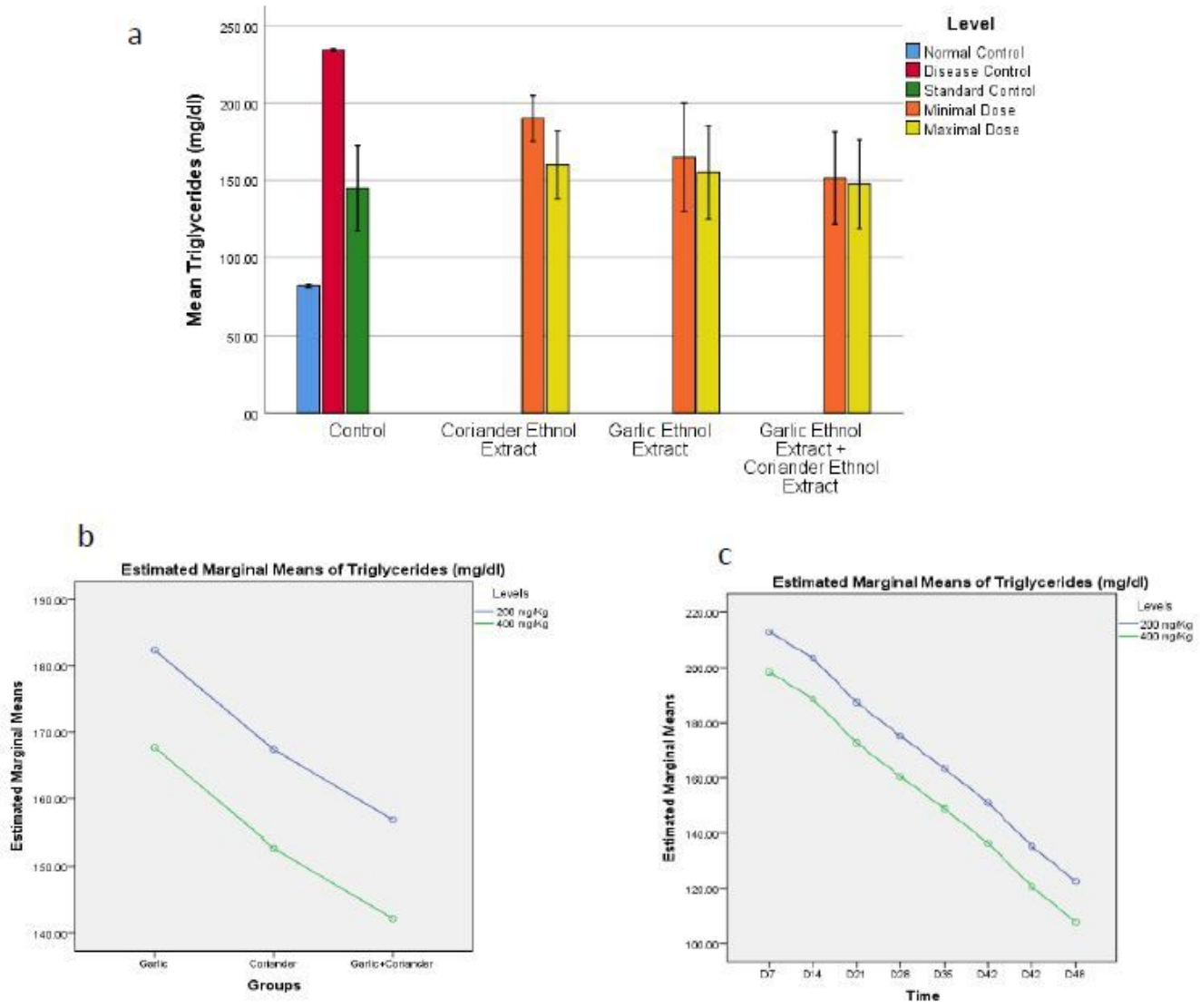

Figure 11

a): Effects of Medicinal herbs on Triglycerides (mg/dl) in diabetic mice(s). b) Estimation of comparative effectiveness of extracts on Triglycerides (mg/dl) over two dose levels. c) Estimation of comparative effectiveness of extracts on Triglycerides (mg/dl) over eight equal time intervals.

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