ISSN:2249-1236
Rawoof khan.G et al. IJRRPAS, (2017) Vol.7 Issue.1 Pg:-1450-1457
International Journal of Research and Reviews in Pharmacy and Applied sciences

Nephroprotective and Hepatoprotective Activity of Cold-Pressed Extract of *Nigella Sativa L .Oil* in Rat

Rawoof khan.G*, Saeed Ahmed khan[1]

*Department of Pharmacology and Toxicology, Dubai Pharmacy College for Girls, Dubai, UAE
[1]Department of Pharmaceutical chemistry and Natural product, Dubai Pharmacy College for Girls, Dubai, UAE

Abstract:
Herbal medicines are use of herbs for their therapeutic (or) medicinal values for its medicinal. *Nigella sativa Linn*. is family of Ranuculaceae, commonly known as the black cumin seed. *N. sativa* seeds oil were used for the extraction of their oil by way of cold press method using the machine and filter to determine the physicochemical properties. Eight groups were employed in the present study and four groups for nephrotoxicity and four groups heptotoxicity comprised six rodent rats. Nephrotoxicity induce by Gentamicin (80 mg/kg/day) intraperitonially was administered for 10 days in rats for four groups. Heptotoxicity induced by 1g/kg of paracetamol for four groups of animals after fasting. After 28 days treatment, all groups are anesthetized and blood was collected by retro orbital puncture and serum was separated for the estimation of hepatic enzymes alanine aminotransferase (ALT or GPT) activities, alkaline phosphates (ALP) activities, AST, total cholesterol, Gamma-GT, Triglyceride, blood urea nitrogen, serum creatinine, uric acid and total protein and sacrificed the all groups animals removed liver and kidney were excised out and fixed for histopathology studies. Statistical analysis was performed as the mean± standard deviation (SD).
Keywords: Nephroprotective, Heptoprotective, *Nigella sativa oil,* blood urea nitrogen, alanine aminotransferase

Aim and Objective:
Herbal medicines are use of herbs for their therapeutic (or) medicinal values for its medicinal. *Nigella sativa Linn*. is family of Ranuculaceae .The use of plant extracts can be great significance in therapeutic treatments.
In this study we investigate the effect of *Nigella sativa oil* on hepatoprotectivity and nephroprotectivity in rat.

Introduction:
The world health organization (WHO) estimates that 4 billion people 80% of the world population presently use herbal medicine for some aspects of primary health care. WHO notes that of 119 plants derived pharmaceutical medicines, above 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures?
Substances derived from the plants are used today for the treatment of some diseases. Like heart diseases, high blood pressure, pain asthma and other problems.
Plant is still a potent source of therapeutic agents they are popularized due to their effectiveness, easy availability, low cost and comparatively being devoid of serious toxic effects. For example: the drug like *Nigella sativa oil* has the activities like antimicrobial, analgesic, anti-inflammatory.
N*igella sativa* Linn., is an annual herb that belongs to the botanical family of Ranuculaceae, commonly known as the black cumin seed, and there are many common terms from Roman, Russia, India and Pakistan. *Nigella sativa oil* oil was reported to attenuate renal injury in a self-sensitive hypertension model[1]. Further, Black seed oil has been shown to protect against renal injury in rat model of obesity

and hypertension[2]. In addition, Black seed oil protected diabetic mice by reversing podocyte injury[3]. Moreover, Black seed oil has been shown to reverse high glucose induced loss of mesangial cells[4].

Nigella sativa oil contains an abundance of conjugated linoleic (18:2) acid, thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, damascenine, and tannins, campesterol, stigmasterol. It contain more than 30% of fixed oil and 0.4 – 0.45 % wt/wt of volatile oil. The volatile oil contains 18.4 – 24% thymoquinone (TQ) and 46% many monoterpenes such as p-cymene and α– piene (Nickavar B. et al., 2003).[5] The volatile oil has antioxidant properties (Burits M. and Bucar F., 2000).[6] Though the *in vitro* potential of protecting renal cells against gentamicin-induced renal toxicity has been demonstrated the *in vivo* renoprotective effect of Black seed oil against gentamicin induced nephrotoxicity is not known.[7]

Material and Methods:

Plant material and extraction procedure

The black seeds were purchased from a Sudan and classified according to the family and species identify by Dr. Naglaa gamil, professor, Dubai Pharmacy College, Department of pharmaceutical chemistry and natural products, Dubai, UAE. The seeds where cleaned and poured in cold compressor machine and extract the oil by cold pressing method collect the oil from the machine and filter it and concentrated oil get.

Detection of chemical compounds in ethanolic extract

Chemical detection was carried out using different reagents as mentioned in to determine the quality of active compounds exists in crude extract.

Detection of sesamin using (HPLC)

Quality and quantity analysis was done by HPLC technique analysis using C-18 column, 50 × 4.6 mm I.D column, the mobile phase used was 1% phosphate buffer(pH =4.5): acetonitrile:water (60:40), and the flow rate was 1ml/min at 264 nm. The volume of injected extract was 20µl.The peak area was calculated and compared with standared.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was done for the presence of carbohydrates, proteins, saponins, alkaloids, flavonoids, tannins, tri-terpinoids and phenolic compounds according to the procedure described in “Textbook of Practical Pharmacognosy” by C.K. KOKATE.[8]

Experimental Design:

Animals:

Forty eight either rodent rat for weighing 25-30gm was obtained from Dubai pharmacy college, Dubai, UAE. Twenty four animals grouped for hepatotoxicty and twenty four animals separately grouped for nephrotoxicity. All the animals were kept under constant environmental conditions with a 12/12 light-dark cycle and temperature of 23±2◦C, fed with standard granulated chow, and given drinking water *ad libitum*. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care. The experimental protocol (Reg no: DPC/AEC/2016-17/ 45) was approved by Dubai Pharmacy college Animals Ethic Committee.

Hepatoprotective activity[9]

Each groups consisting of 6 animals. All animals were fasted over night before the experiment and administrated 1g/kg of paracetamol for all group of animals after 24 hours the rats treated as per Group 1: animals received single dose of (1g /kg) body weight of paracetamol by injected intraperitoneal followed by normal saline orally. (Control)

Group 2: animals received STD drug ascorbic acid 100mg/kg administered orally daily
Group 3: animals received 5ml/kg administered oil of *Nigella sativa* orally daily.
Group 4: animals received 10ml/kg administered oil of *Nigella sativa* orally daily
After 28 days treatment, all group animals are sacrificed and dissected liver and weighed on electronic balance and fixed for histopathology studies.

Biochemical tests:
Blood were drawn via retinol orbital puncture technique from anesthetized rat and were then centrifuged at 3000 rpm for 10 min to separate the serum. The serum was stored at -40°C until enzyme assays were carried out. The hepatic enzymes alanine aminotransferase (ALT or GPT) activities, alkaline phosphates (ALP) activities, AST, total cholesterol, Gamma-GT, Triglyceride, were measured by (using Diasys Liver Enzyme Kits) and serum total protein concentration were estimated using Reitman and Frankel methods [10],Kind and kings method [11], Evelyn and Malloy method [12] and Biuret method[13] respectively.

Nephroprotective Activity[14]
Four groups were employed in the present study and each group comprised six rodent rats. Gentamicin (80 mg/kg/day) intraperitonial was administered for 10 days to induce experimental nephrotoxicity in rats for all groups.
Group 1: animals served as control was injected with gentamicin (80 mg/kg/day) intraperitonial standard food and water throughout the experiment.
Group 2: animals was injected with gentamicin (80 mg/kg/day) intraperitonial, standard drug ascorbic acid 100mg/kg/day
Group 3: animals administered with *Nigella sativa oil* (5ml/kg/day) orally and injected with gentamicin (80 mg/kg/day) intraperitonial
Group 4: animals were *Nigella sativa oil* (10ml/kg/day) orally and the treatment was started days prior to gentamicin administration. The treatment periods for all these groups were 28th days. On 28th day under mild ether anesthesia, blood was collected by retro orbital puncture and serum was separated for the estimation of blood urea nitrogen, serum creatinine, uric acid and total protein. Then the animals were sacrificed and kidneys were removed for histopathology studies.

Biochemical estimation:
Estimation of Serum Creatinine:
The serum creatinine concentration was estimated by alkaline picrate method[15] using the commercially available kit. Briefly 2.0ml of picric acid reagent in a tube was added to 0.2ml of serum for deproteinization of specimen, which was mixed well and centrifuged at 3000 rpm to obtain a clear supernatant. 100 µl of buffer reagent was added to 1.1 ml of supernatant, 0.1 ml of standard creatinine and 0.1 ml of distilled water to prepare test, standard and blank, respectively. 1.0 ml of picric acid reagent was added to blank and standard. The test tubes were mixed well and kept at room temperature for 20 minutes. The alkaline picrate reacts with creatinine to form the orange colored complex, which was read at 520 nm spectrophotometrically.

Estimation of Blood Urea and Urea Nitrogen:
The blood urea was estimated by Berthelot method[16] using the commercially available kit. 1000 µl of working reagent-1 containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the tubes were mixed well and incubated at 38 oC for 5 min. then 1000 µl of reagent-2 containing alkaline buffer, was added to all the test tubes, which are incubated at

38 oC for 5 min. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite and nitropruside to yield indophenol, a blue-green colored compound. The intensity of the color produced is directly proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm. The blood urea was calculated using the formula:

Estimation of Protein in Urea:

The protein urea was assessed by pyrogallol red method [17] using the commercially available kit. 1000 µl of reagent (pyrogallol dye) was added to 10 µl of urine sample, 10 µl of standard protein and 10 µl of purified water to prepare test, standard and blank respectively. All the test tubes were mixed and incubated at 38 oC for 10 min. The absorbances of test and standard samples were noted against blank at 600 nm spectrophotometrically. When the pyrogallol red-molybedate complex binds to basic amino group of protein molecules, there is a shift in reagent absorbance. The absorbance is directly proportional to protein concentration present in the sample. The urinary protein was calculated using the formula

Histopathological Examination The liver and kidneys were sectioned longitudinally into two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. These sections were stained with hemotoxylin and eosin and were observed under a computerized light microscope.

Statistical Analysis All values were expressed as mean ± S.D. The data obtained from various groups were statistically analysed using oneway ANOVA. The P value of less than 0.05 was considered statistically significant.

Results:

Hepatoprotective effect:

Paracetamol (1g /kg) given intraperitoneal showed hepatotoxicity

Experiment after 28 days has evident from biochemical parameter of study. The levels of liver enzymes in rat group AST, ALP, ALT where increased in the control animals and the test *Nigella sativa oil* (5ml/kg) show the less significant effect of liver enzyme in the treatment period compare to test group *Nigella sativa oil* (10ml/kg) was more significant effect of the liver enzyme compare to the standard drug ascorbic acid (100mg/kg) group of animals. Shown in (table 2)

Nephroprotective effect:

Effect of Black seed oil on Serum Creatinine, Blood Urea Nitrogen, demonstrates the effect of Black seed oil about the serum creatinine, blood urea and urea nitrogen. There was marked increase in serum creatinine was noted in gentamicin administered albino rats as compared to normal albino rats. In addition, the blood urea and nitrogen urea were noted to be increased in gentamicin-administered albino rats. However, concomitant administration of Black seed oil in two doses (5ml/kg&10ml/kg) significantly reduced gentamicin-induced elevated levels of serum creatinine and blood urea and urea nitrogen in albino rats shown in (table 1).

Effect of Black seed oil on Serum Uric acid:

Fig 3 show the effect of black seed oil about the serum uric acid level its marked that increase of uric acid level in the gentamicin administrated control animal and the treated with black seed oil animal shows decrease in the uric acid level . It show that the kidney is healthy in the both test dose (5ml/kg&10ml/kg) shown in (table 1).

Effect of Black seed oil on total Protein urea:

FIG. 4 shows the effect of Black seed oil in gentamicin administered albino rats and it showed marked induction of protein urea as compared to normal albino rats. However, the concurrent administration

of Black seed oil (5ml/kg&10ml/kg) significantly reduced the incidence of protein urea in albino rats administered gentamicin(tabel 1).

Histopathological Studies:

Hepatoprotective activity:

The results of histopathological changes of the experiment are presented in figure 2.In group C (5ml/kg), hepatocytes contained multiple small vacuolation at many places. Hepatocytes appeared swollen with fragmented or granular cytoplasm.

Group D (10ml/kg), showed diffuse vacuolar degeneration with swollen hepatocytes. Fragmentation of cytoplasm, cytoplasmolysis and total disappearance of cells were observed

Neproprotective activity

Severe disruption of glomerular capillaries in A and recovered in B, C&D groups noticed shown in figure1

Discussion:

Nigella sativa seeds are orally ingested by people as condiment or additive in food dishes. Patients with gastrointestinal disorders ingest seeds mixed with honey. Our study was undertaken to demonstrate the effect of volatile oil against toxicity.

As a result, the volatile oil of *Nigella sativa* and may be its constituent, thymoquinone, can reduce the pain and may be used as an analgesic drug.

Protective effect of *Nigella sativa* oil on liver function against paracetamol induced acute toxicity, paracetamol hepatotoxicity manifested biochemically by elevation of serum levels of liver enzyme. Paracetamol caused a significant increase in serum GPT and total protein compared with results. The significantly increase of Alanine aminotransferase (ALT or GPT), Aspartate aminotransferase (AST or GOT) enzymes in group treated with paracetamol indicated to the parenchymal hepatotoxic effect induced by paracetamol.This increase maybe cause by the damage of liver cell membrance by free radicals and release of these enzyme into blood.

The study of the liver histopathology showed marker reduction in sinusoidal dilation ,midzonal necrosis after treatment with *Nigella sativa* extract and this indicator to hepatoprotective action against paracetamol These results due to the time that need for the liver to pretreatment and return to his function again .In present study the level of liver enzyme stile significantly increased after treatment with *Nigella sativa* because the period of treatment only one day which mean it need more time to return the function.

Nephroprotective is elevated levels of serum creatinine, blood urea nitrogen uric acid, total protein, total calcium. The ability of the kidney to filter creatinine (a non-protein waste product of creatinine phosphate metabolism) is reduced during renal dysfunction as a result of diminished glomerular filteration rate. Thus, the increase in serum creatinine level is an indication of renal dysfunction. Moreover, the elevated levels of blood urea nitrogen and uric acid occur during renal dysfunction. The incidence of proteinuria is associated with glomerulosclerosis and tubulointerstitial fibrosis. In the present study, the gentamicin administration in albino rats increased the level of serum creatinine. In addition, the blood urea nitrogen and uric acid levels were increased in gentamicin administered albino rats as compared to normal albino rats. Furthermore, the elevated level of urea protein was noted in gentamicin-administered albino rats as compared to normal albino rats. These results suggest the development of renal damage and renal dysfunction in albino rats administered gentamicin.

These results suggest that *Nigella sativa* oil has an ability to prevent gentamicin-induced nephrotoxicity in albino rats.

ISSN:2249-1236
Rawoof khan.G et al. IJRRPAS, (2017) Vol.7 Issue.1 Pg:-1450-1457
International Journal of Research and Reviews in Pharmacy and Applied sciences

Conclusion:

The active constituent of the volatile oil may be thymoquinone, has an analgesic effect to reduce pain, the volatile oil was dose dependent, but we should give a small and accurate dose of it to prevent toxicity. From the results of this study it can be conclude that treatment with low lethal dose of paracetamol caused damage to the liver but when administered the oil of *Nigella sativa* after injection with paracetamol may be protective the liver but it need more time than one day. The present study investigated the effect of *Nigella sativa* oil, a lipophilic statin, in gentamicin-induced nephrotoxicity in albino rats. It suggests that *Nigella sativa* oil has an ability to halt the development of gentamicin induced nephrotoxicity in albino rats. Black seed oil may prevent gentamicin nephrotoxicity in albino rats by reducing renal damage.

Figure 1: Histopathology studies of Nephroprotective effect of *Nigella sativa oil* in gentamicin induced animals kidney tissues

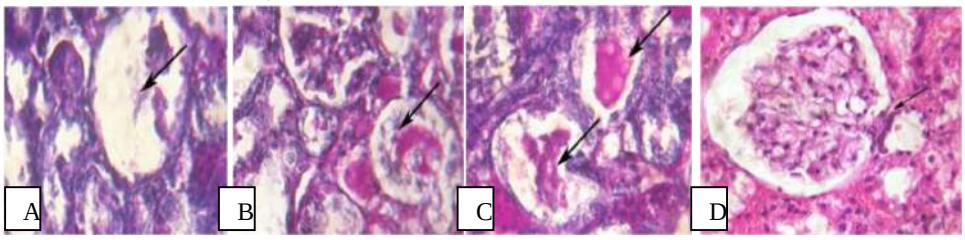


Figure1: A: Control B: standard C: Nigella sativa oil 5ml/kg D: Nigella sativa oil 10ml/kg gentamicin induced kidney: Severe disruption of glomerular capillaries in A and recovered in B, C&D groups noticed

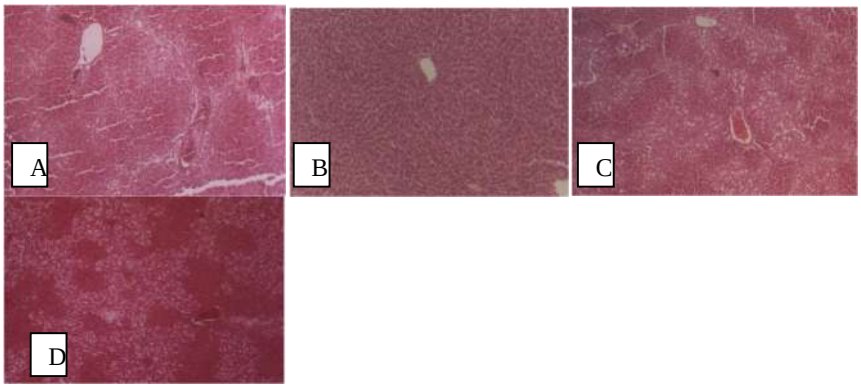
Table 1: Nephroprotective Effect of *Nigella sativa oil* in gentamicin induced animals

Group	BUN mg/dl	Creatinine mg/dl	Uric Acid mg/dl	Total Protein g/dl
Control	37.3±1.00	0.8±0.22	2.35±0.47	18.5±0.05
Std(Ascorbic Acid 100mg/Kg)	14.2±1.24***	0.23±0.04**	1.33±0.83**	13.9±0.43**
Nigella Sativa Oil(5ml/kg)	24.7±2.25	0.37±0.14*	1.35±0.41*	13.3±1.56**
Nigella Sativa Oil(10ml/kg)	17±2.91***	0.24±0.01**	1.17±0.61***	11.3±1.49***

*Values are represented as mean ±SD, where n=6, ***P<0.001 as compare to normal control, **p<0.01 as compare to control.BUN (blood urea nitrogen), STD (standard*

ISSN:2249-1236
Rawoof khan.G et al. IJRRPAS, (2017) Vol.7 Issue.1 Pg:-1450-1457
International Journal of Research and Reviews in Pharmacy and Applied sciences

Figure 2: Histopathology studies of Hepatoprotective effect of *Nigella sativa oil* in paracetamol induced animals liver tissues



A: Liver section, control group, showing diffuse areas of vacuolar degeneration and centrilobular necrosis with mononuclear cell infiltration, B: Liver section, standard drug group, showing mild hepatocyte vacuolation , C&D :Liver section, rats treated with Nigella sativa oil showing massive vacuolar degeneration and centrilobular necrosis

Table 2: Hepatoprotective Effect of *Nigella sativa oil* in paracetamol induced animals

Group	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Total protein	Gamma GT
Control	207.5±1.43	525± 4.9	180± 1.79	100.5±1.92	67.5±1.3	9.5± 0.14	8.5±2.19
Std(Ascorbic Acid 100mg/Kg)	158.6±0.24 *	109.2±0.53**	60.7± 1.82***	57.2±3.13**	35±2.77 **	7.42±1.18**	2.5±3.69**
Nigella Sativa Oil(5ml/kg)	183.3±2.40	170.7±1.88**	186±3.04	79 ±2.75 **	73±1.97	7.90±0.62*	5.6±2.90
Nigella Sativa Oil(10ml/kg)	141.5± 2.88**	185± 1.0 **	121.2±2.84*	80.25±1.87*	47±3.07*	7.07±1.29**	3.0± 0.95*

*Values are represented as mean ±SD, where n=6, ***P<0.001 as compare to normal control, **p<0.01 as compare to control. AST (Aspartate transaminase), ALP (alkaline phosphates), ALT (alanine aminotransferase), STD (standard)*

Reference:

1. Vieira JM, Rodrigues LT, Mantovani E, Delle H, Matter AL, et al. Statin monotherapy attenuates renal injury in a salt-sensitive hypertension model of renal disease. Nephron physiol 2005; 101: 82-91.
2. Knight SF, Yuvan J, Roy S, Imig JD. Simvastatin and tempol protect against endothelial dysfunction and renal injury in a model of obesity and hypertension. Am J Physol 2010; 298: 86-94.
3. Wei P, Grimm PR, Settles DC, Balwanz CR, padanilam BJ, Sansom SC. Simvastatin reverses podocyte injury but not mesangial expansion in early stage type 2 diabetes mellitus. Ren Fail 2009; 31: 503-13
4. Lin CL, Tung CW, Huang WJ, Chang PJ, Yang JT, Wang JY. Simvastatin reverses high glucose-induced apoptosis of mesangial cells via modulation of Wnt signaling pathway. Am J Nephrol 2008; 28: 290-7.
5. Nickavar B, Mojab F, Javidnia K, Amoli MA. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z. Naturforsch. 2003; 58: 629–631.
6. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Res. 2000; 14: 323-328.
7. Antoine DJ, Srivasthava A, Pirmohamed M, Park BK. Statin inhibits aminoglycoside accumalation and cytotoxicity to renal proximal tubule cells. Biochem pharmacol 2010; 79: 647-54.
8. Kokate CK, Purohit AP, Gokhale SB. Practical Pharmacognosy. 2nd ed. Mumbai: Nirali Prakashan, Pune; 1994.
9. Rao PG, Rao G, Ramnarayan K, Srinivasan KK. Effect of hepatogard on paracetamol-induced liver injury in male albino rats. Indian Drugs 1993; 30:41-6.
10. Reitman S, Frankel SA. Chlorimetric method for determination of pharmacological evaluation of plant material. England: John Wiley; serum glutamic oxaloacetic and glutamic pyruvate transaminases. Am 1996.J Clin Pathol 1957; 28:56-63. 18. Moss DW, Butterworth PJ. Enzymology and medicine. London: Pitman
11. Kind PRN, King EJ. Estimation of plasma phosphatases by Medical; 1974. determination of hydrolyzed phenol with antipyrene. J Clin Pathol 19. Das KK, Dasgupta S. Influence of ascorbic acid on acid and alkaline1954; 7:322-30. phosphatase activities in some metabolically active tissues of aspirin
12. Malloy HT, Evelyn KA. The determination of bilirubin with treated rats. Indian J Physiol Pharmacol 1997; 41:421-3.photometric colorimeter. J Biol Chem 1949; 4:481-90.
13. Annino JS. Clinical chemistry, principle and procedure. 4th ed. Boston: Little brown and Company; 1976.
14. Upaganlawar A, Mamta F,Shivkumar R, Balaraman R. Modification of biochemical parameters of gentamicin nephrotoxicity by coenzyme Q10 and green tea in rats. Indian J Exp Biol 2006; 44: 416-418.
15. Bones, R.W. etal. (1945) J. Biol. Chem. 158, 581.Procedure Toro, G. etal. (1975) Practical Clinical Chem. P: 154.
16. Chaney, A.L and Marbach, E.P. (1962) Clin. Chem.8.,130
17. Dilena, B.A. et al., Six methods for determining urinary protein compared. Clin. Chem., 29, 553– 557 (1983)