THE PROTECTIVE ROLE OF PROPOLIS ON GENTAMICIN-INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

The present work investigate the effect of propolis on gentamicin induced nephrotoxicity in rats. Male Wistar rats were divided into 4 groups; saline, gentamicin (100 mg/kg b.w, i.p., intraperitoneally) for 8 consecutive days, propolis PR (200 mg/kg b.w., p.o.), for 14 consecutive days, propolis 14 days and concurrently with gentamicin for 8 days. Blood urea, serum creatinine, plasma malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) activities and microscopic examination of kidney were performed after the treatment.The results showed that gentamicin treatment caused nephrotoxicity evidenced by marked elevation in blood urea and serum creatinine. Blood urea and serum creatinine were increased in animals treated with gentamicin compared to saline-treated animals. Co-administration of propolis with gentamicin ameliorate blood urea and serum creatinine. GEN administration to rats increased renal MDA and decreased SOD and CAT activities. PR administration with GEN injections significantly decreased MDA, and increased SOD and CAT activities when compared with GEN group.

KEYWORDS: Propolis ; Gentamicin; Reactive Oxygen Species; Renal toxicity.

INTRODUCTION

Neprotoxicity induced by gentamicin (GEN is a complex phenomenon characterized by an increase in blood urea and serum creatinine

concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure (Al-Majed et al., 2002). Although the pathogenesis is still not well understood, the toxicity of GEN in the kidney seems to relate to the generation of destructive reactive oxygen species (ROS) in these cells (Reiter et al., 2002). ROS have been implicated in a wide range of biological functions, but they can express both beneficial and highly toxic effects on cellular homeostasis (Mates, 2000). A large body of in vivo and in vitro evidence indicates that ROS are important mediators of GEN-induced nephrotoxicity (Kopple et al., 2002). ROS have been proposed as a causative agent of cell death in many different pathological states as well as, in glomerular disease (Smetana et al., 1988), in renal ischemia and reperfusion injury (Longoni et al., 2002), and in various models of toxic renal failure (Piotrowski et al., 1996). Several studies have demonstrated that various agents including melatonin (Ozbek et al., 2000), vitamin E, lipoic acid (Al-Majed et al., 2002), ginkgo biloba extract (Maldonado et al., 2003) can prevent GEN-induced renal damage.

Propolis, a resinous substance that honeybees produce by mixing their own waxes with resins collected from plants, is used as a sealant and sterilizing agent in honeybee nests (Nakajima et al., 2007). Propolis has been used also as a folk medicine in many countries for its particular biological properties in the treatment of cancer and as an antioxidant, anti-microbial, anti-inflammatory and antibiotic agent (Marcucci, 1995 and Banskota et al., 2002). The flavonoids, aromatic acids and phenolic compounds are responsible for the most biological and pharmacological activities of propolis (Vennat et al., 1995). The current work aims to study the effect of propolis against gentamicin-induced nephrptoxicity in albino rats.

MATERIALS AND METHODS

Animals

Forty male albino rats, weighing 200 - 250 g were used in this study; they were housed under conditions of controlled temperature and were

fed standard rat chow. Animals were divided into four groups of 10 animals each. First group includes animals that received only saline throughout the course of the experiment. Animals of the second group received daily intraperitoneally injection of gentamicin (100 mg/ kg/b.w) for eight days, (Abdel-Gayoum et al., 1994). Animals of the third group were given gentamicin (100mg/kg b.w) intraperitoneally for eight days in addition to 200 mg/kg propolis (p.o.) for 14 days. Group four,animals of this group received propolis (200 mg/kg b.w) per orally for 14 days .

At the end of experiment, rats were killed and the kidneys quickly removed, decapsulated and divided equally into two longitudinal sections. One of these was placed in 10% neutral formalin solution for routine histological examination by light microscopy. The other half was placed in liquid nitrogen and stored at −85^{o}C until assayed for MDA, SOD and CAT activities. Trunk blood was extracted to determine the serum levels of blood urea and Creatinine. For these studies, PR (natural product) was dissolved in saline. PR was administered at a dose level of 200mg/kg b.w which was reported that completely blocks production of ROS (Ozyurt et al., 2001).

Biochemical investigations:

Serum Creatinine level was determined using Olympus Autoanalyzer (Olympus Instruments, Tokyo, Japan) and blood urea concentration was determined by GLDH-Kinetic method, using Beck man Spectrophotometer.

Renal antioxidant enzyme activities:

SOD activity determination was based on the production of H_2O_2 from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described (Goering et al., 2002). The product was evaluated spectrophotometrically at 560 nm. CAT enzyme activity was determined according to Aebi's method (Aebi , 1974). The principle of the assay is based on the determination of the rate constant or the H_2O_2 decomposition rate at 240 nm.

Malondialdehyde (MDA) level:

MDA level in the homogenates were assayed spectrophotometrically at 535 nm according to Mihara and Uchiyama (1978). A standard calibration curve was drawn by using 1,1,3,3-tetramethoxypropane.

Histological observation

For light microscopic evaluation, portions of each kidney were fixed in 10% neutral – formalin, dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. Tissue sections of 6 μm were stained with hematoxylin and eosin.

Statistical analysis

Kidney MDA, SOD, CAT, serum urea and Creatinine levels were analyzed by one-way ANOVA. Posthoc comparisons were done using Tukey's tests. Differences were considered significant at $P < 0.05$. Results are expressed as mean±SD.

RESULTS

1-Effect of PR on GEN-induced changes in serum parameters.

Table 1 showed that serum levels of blood urea and creatinine in the GEN-treated animals are significantly higher than control group [27.4 ±2.3 versus 58 ±3.7; 0.35±0.02 versus 0.76 ±0.02, respectively].

Sera of animals treated with both GEN and PR showed significantlyreduced urea and creatinine levels when compared with animals of GEN group [58 ±3.7versus 32.6 ±+4.1; 0.76 ±0.02versus 0.44 ±0.03, respectively]. There were no statistically differences between control and PR treated groups.

2- Effect of PR on GEN-induced changes in kidney tissue enzymes, lipid peroxides.

Data in Table 1. GEN-induced acute renal failure manifested by a significantly increased kidney MDA level [61.44 ±7.40versus 100.79±2.56] and significantly decreased SOD,CAT activities [158.1±4.49 versus 137.71 + 2.41 and 5.83±0.28versus 1.74±0.31, respectively]. PR administration with GEN injections caused significant decrease in MDA[100.79±2.56versus 71 + 4.83] and increased in SOD, CAT activities [164.36±7.90versus 164.36 ±7.90; and 1.74±0.31versus 4.02±0.23; respectively] in kidney when compared with GEN. There were no statistical differences between control and PR-treated groups.

Table 1: Effects of gentamicin (GEN) administration on rats with or without propolis (PR) on blood urea and creatinine levels,kidney MDA level and SOD&CAT activities.

Parameters	Control	GEN	GEN+ PR	PR
Blood urea (mg/dL)	27.4 ±2.3	58 ±3.7[a]	32.6 ±4.1[b]	27.7 ±1.9
Creatinine (mg/dL)	0.35± 0.02	0.76 ±0.02[a]	0.44 ± 0.03[b]	0.37±0.03
MDA (nmol/gtissue)	61.44 ±7.4	100.79±2.56[a]	71 ± 4.83[b]	66.32± 3.0
SOD (U/gm tissue)	158.1±4 .49	137.71±2.4 1(a)	164.36±7.9 0(b)	154.45±5 .61
CAT (k unit/ml)	5.83± 0.28	1.74± 0.31[a]	4.02± 0.23[b]	5.98±0.3 4

Data are shown as means ± SD
([a]) P < 0.05 versus control group.
([b]) P < 0.05 versus GEN group.

3- Effect of PR on GEN-induced morphological changes in kidney tissue:

The histological results are showed some morphological changes in kidneys. Kidneys of animals of control group showed normal kidney structure (Fig. 1a). On the other hand, there were not any microscopical differences between the control and only PR-treated groups. GEN-treated rats. More extensive and marked tubular necrosis was seen (Fig. 1b). In

the GEN+ PR-treated rats, sparse tubular changes were observed (Fig. 1c). PR apparently reduced kidney tissue damage.

DISCUSSION

The nephrotoxicity of aminoglycoside antibiotics, and specially that of the most commonly used compound, gentamicin, is well documented (Cuzzocrea et al., 2002; Al-Majed et al., 2002). Several studies have reported that oxygen-free radicals are considered to be important mediators of GEN-induced acute renal failure (Karahan et al., 2005). Accordingly, among the main approaches used to ameliorate GEN - induced nephrotoxicity is the use of agents with powerful antioxidant properties. Several recent studies have reported that the propolis or its components may be useful in ameliorating signs of GEN nephrotoxicity (Parlakpinar et al., 2005). In this study, the antioxidant properties of PR to prevented the nephrotoxicity by improving histopathological changes. Several dosages have been reported for GEN administration. In the present study, acute nephrotoxicity was created by injecting GEN (100 mg/kg b.w i.p.). In the present study, PR is a potent antioxidant and free-radical scavenger on the renal damage and oxidative injury induced by GEN.

Plasma creatinine concentration is a more potent indicator than the urea concentration in the first phases of kidney disease. Furthermore, urea concentration begins to increase only after parenchyma tissue injury (Gilbert et al., 1989). In this study plasma creatinine and urea levels were higher ($p<0.05$) in the GEN group when compared with the control group. So, the elevation in blood urea & creatinine levels in GEN treated rats is considered as marker of renal dysfunction. This result is in agreement that reported by Kopple et al. (2002), Parlakpinar et al. (2005) .

In the current study, GEN induced oxidative stress which results in lipid peroxidation causing increase in MDA levels and decrease in antioxidant enzymes like catalase and superoxide dismutase CAT is a hemoprotein which catalyses the reduction of hydrogen peroxide and

protects the tissues from highly reactive hydroxyl radical (Rajasekaran et al., 2005). The reduction in the activities of this enzyme could reflect the adverse effect of GEN. Furthermore, the propolis treatment prevented depletion of CAT activity induced by gentamicin. The protective effect might be due to the ability of propolis to inhibit hydrogen peroxide-induced oxidative injury in renal cell line (Parlakpinar et al., 2005).

In the current study, GM induced oxidative stress which results in decrease in antioxidant enzymes like catalase and superoxide dismutase (SOD). There are some experimental data suggesting that nephrotoxic drugs may also change levels of MDA, glutathione peroxidase (GSHPx), CAT, SOD, GSH, BUN and Cr (Ozbek et al., 2000) which are commonly used to monitor the development and extent of renal tubular damage due to oxidative stress.

Thus, the preventive effect of propolis on the gentamicin induced decrease in the activity of superoxide dismutase (SOD) and CAT could be contributed to the restoration of markers of renal tubular injury. It seems reasonable to assume that propolis is able to suppress nephrotoxicity in kidney as it was demonstrated in studies with gentamicin (Parlakpinar et al., 2005) and (Vardi et al., 2005), amikacine (Parlakpinar et al., 2005) and doxorubicin (Yagmurca et al., 2005).

The effects of CAPE on GEN-induced renal changes using both biochemical determinations and the morphology of the kidney using light microscopy (Parlakpinar et al., 2005).

In this study, it has been shown that GEN, at 100 mg/kg, significantly increased the level of lipid peroxidation products (MDA) suggesting the involvement of oxidative stress.

On the other hand, the effect of GEN and elevation of the lipid peroxidation product, MDA, were reduced by propolis treatment which are in line with various previous reports. The present results showed that propolis decreases lipid peroxidation possibly by its antioxidant activity.

Parlakpinar et al., (2005) reported a protective effect of propolis on circulating lipids in plasma and on lipid peroxidation products in alcohol and on polyunsaturated fatty acid induced toxicity. Lipid peroxidation, mediated by oxygen-free radicals, is believed to be an important cause of destruction and damage to cell membrane. Malondialdehyde is formed during oxidative degeneration and is accepted as an indicator vancomycin-induced injury and that supplementation with CAPE may be helpful in reducing vancomycin nephrotoxicity (Iwamoto et al., 2003).

Histopathological results demonstrating structural changes in renal tissue of aminogylcoside antibiotics such as GEN were reported by some researchers (Kumar et al., 2000; Al-Majed et al., 2002; Ateşşahin et al., 2003; Polat et al., 2006; Sayed-Ahmed and Nagi, 2007). The histopathological results in this study are in rapport with these reported studies. In the present study, Glomerular and tubular epithelial changes were considerably mild in the groups treated with PR. The histopathological results in connection with protective effects of PR or its component for induced nephrotoxicity of aminoglycosides including GEN were in agreement with other reports (Parlakpinar et al., 2005). We think that, morphological changes in kidneys were because of GEN injection, but these changes tended to be considerably mild in GEN plus PR injection.

In conclusion, the GEN -induced nephrotoxicity may be related with oxidative damage. Co-administration of PR decreased the harmful effects of GEN both by inhibiting free-radical formation and by restoration of the antioxidant systems. Further investigations on the mechanism of action of PR are required and may have a considerable impact on future clinical treatments of patients with renal failure.

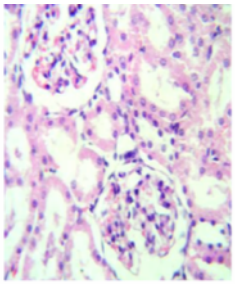

Figure 1a. Tubules appear normal in the control group. H–E X 66.

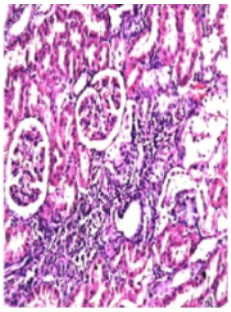

Figure 1b. tubular necrosis is observed in the GEN-treated group. H–E X 66

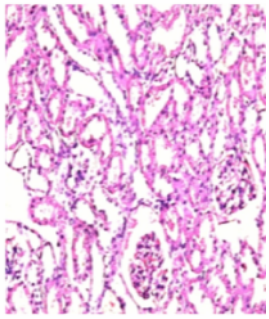

Figure 1c. Tubules show slight histological changes in the GEN+ PR -treated group .The tubules revealed normal. H–E X 66.

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الدور الواقى لصمغ النحل(البروبوليس) على التسمم الكلوى المحدث بالجنتاميسين فى الجرذان البيضاء.

أبراهيم حلمى ، طارق سالم ، أيمن المغاورى، خالد بسيونى،ماجدولين قابل

قسم البيولوجية الجزيئية-معهد الهندسة الوراثية والتكنولوجيا الحيوية-جامعة المنوفية.

الهدف من البحث تحديد تأثير صمغ النحل(البروبوليس)على التسمم الكلوى المحدث بالجنتاميسين فى ذكور الجرذان البيضاء. اشتملت الدراسة على عدد اثنان و ثلاثون (٣٢) فأراً أبيض قسمت إلى خمس (٤) مجموعات كل منها يتكون من ثمانية (٨) فئران.المجموعة الأولي :تتعايش الجرذان بهذه المجموعة تحت الظروف العادية من حيث التغذية والشراب المجموعة الثانية :تم حقن الجرذان في تجويف البطن بالجنتاميسن بجرعة مقدارها ١٠٠ مليجرام/ كليوجرام يومياً لمدة ثمانية أيام متتالية المجموعة الثالثة :تم إعطاء معلق صمغ النحل(البروبوليس)عن طريق الفم بجرعة ٢٠٠ مليجرام/كليوجرام يوميا لمدة ١٤ يوم متتالية ثم حقن الجرذان في تجويف البطن بالجنتاميسن بجرعة مقدارها ١٠٠ مليجرام/ كليوجرام يومياً لمدة ٨ أيام متتاليه.المجموعة الرابعة:تم إعطاء معلق صمغ النحل(البروبوليس)عن طريق الفم بجرعة مقدارها ٢٠٠ مليجرام/كليوجرام يوميا لمدة ١٤ يوم متتالية.وقد خضعت الفئران في كافة المجموعات لما يلي :تم أخذ عينات الدم من كل فأر وذلك لقياس:مستوي اليوريا في الدم ومستوي الكرياتين في الدم. تم ذبح الجرذان والحصول على الكلي والكبد وذلك لقياس مستوي الإنزيمات المضادة للأكسدة و دراسة هستوباثولوجية.لوحظ ارتفاع ذي دلالة إحصائية في مستوى اليوريا والكرياتين ومستوى المالونديالدهايد وأيضاً إنزيمات الكبد المعاملة بالجنتاميسن بمفردها إذا ما قورنت بالمجموعة الضابطة وأيضاً لوحظ انخفاض في مستوى الكتاليزو الوسبراوكسيد داى ميوتاز وحدوث تغيرات هستولوجية مرضية في المجموعة الثانية إذا ما قورنت بالمجموعة الأولي.انخفاض مستوي انزيمات الكبد بنسبة تتقارب من المجموعة الضابطة .ارتفاع مستوى مضادات الأكسدة في الدم ثبت أن معاملة الجرذان بالجنتاميسن بعد معاملتها بصمغ النحل(البروبوليس) Post treatment في المجموعة الثالث(PR +GM) تعطي أفضل إمكانية للوقاية من تأثير الجنتاميسن ووضوح الدور الوقائي لصمغ النحل(البروبوليس) .

C-ERB-4 GENE EXPRESION IN CANCER BREAST PATIENTS AND ITS CORRELATION WITH CLINICO PATHOLOGIC PARAMETERS

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ABSTRACT

Breast carcinoma ranks as first malignancy affecting females, contributing 33% of all female cancers. C-erbB-4 a class of oncogenes prevalent in breast cancer and plays a role in cancer development. This research was performed to assess C-erbB-4oncogene amplification by RT- PCR technology. These markers were studied in 50 breast female cancer patients by RT-PCR Technique. Define their relation to various clinical and other prognostic markers and correlate their expression to each other. The results of this study were that C-erbB-4 gene amplification, by RT-PCR was positive in 54% .C-erbB-4 was significantly associated with favorable prognostic markers as absence of lymph nodes involvement and presence of Estrogen and progesterone receptors.

KEYWORDS: Cancer breast, C-erbB-4 -RT-PCR – IHC

INTRODUCTION

Breast cancer is ranking number one after urinary bladder tumors and malignant lymphomas at National Cancer Institute (NCI), Cairo University in Egypt. Breast carcinoma constitutes 33% of all females' cancers in Egypt (El-Bolkainy et al., 2010). Prognostic factors identify patients at higher or lower risk of breast cancer recurrence or death. Useful prognostic factors can be applied broadly to large, heterogeneous

patient groups (Kathy and Miller, 2000). Immunohistochemistry (IHC) techniques are widely used in diagnostic histopathology to help re-differentiate the light microscopically undifferentiated tumors (Ross and Fletcher, 2000). Estrogen and Progesterone are well established steroid endocrine regulators. Estrogen promotes breast epithelial cell proliferation, development as progesterone (Greene et al., 2004). Signal transduction of C-erbB-4 receptor may play an important role in cell growth and differentiation and its expression may be linked to cell differentiation and favorable prognosis in breast cancers (Knowlden et al., 2002). The aim of this investigation was to study of histopathological parameters using light microscopy in breast cancer cases including mitotic Index, immunohistochemical analysis of estrogen, progesterone receptors in breast cancer cases, Assessment of C-erb4 gene amplification and Correlate their expression to each other in newly diagnosed female cancer patients.

MATERIALS AND METHODS

Patients

The present study was performed on 50 diagnosed female breast cancer patients presented to surgical Department, National Cancer Institute (NCI), Cairo University, during the period from 2008 to 2011. Their ages ranged from 27 to 60years old. The fresh tumor tissues were divided into two fragments; one fragment was fixed in 10% neutral buffered formalin (18 to 24 hours), and processed for histological, and immunohistological analysis from paraffin embedded tissues. The second fragment of the tissue was frozen in a dry ice and stored at –80ºC. The latter was used for RNA extraction from cancer breast tissue and adjacent normal tissue as control. One step RT-PCR for C-erbB-4 gene.

Histological diagnosis

For histological diagnosis, tissues were fixed in 4% phosphate-buffered formalin and routinely processed to wax. Paraffin sections (5

μm) were stained with heamatoxilene and eosin and examined with the microscope. (Bancroft and Gamble, 2002).

Immunostaining

Estrogen receptors (ER) and progesterone receptors (PR) were detected using an improved Biotin-Streptavidin Amplified (B-SA) detection system (Taylor and Kledzik, 2002).

RNA Isolation from the tumor extract and RT-PCR analysis

Total RNA was isolated using RNeas total RNA isolation kit supplied by Qiagen (Suo et al., 2002).Gene copy determination using Qiagen one step RT-PCR kit was used in a thermal cycler (Perkin Elmer Cetus).A 100 μl RT-PCR was prepared containing 10 μl of 10x buffer, 200 mM of dNTPs , 1 mM of each primer (erbB4/β-actin), 6 μl 25 mM MgCℓ2, 5 μl Taq DNA polymerase (2.5μ). Then, the volume of 100 μl mix is added to each sample (1μg) DNA. Finally, the samples were loaded in the thermal cycler blocks. Primers (25-mers) were obtained from Gibco BRL, USA. Two primers were used to amplify part of the erbB4 and β-actin as shown (Table 1). PCR was performed in the thermal cycler, Roobycycler gradient 96 Stratagene. Initially, samples were heated for 5 min at 94 oC for denaturation, and then cycled 20 times at 94oC for 1 min, 56 oC for 2 min, and 72 oC for 3 min, followed by a final extension cycle at 72 oC for 5 min.

RESULTS AND DISCUSSION

Histology

Light microscope was used to study the cases of malignant breast lesions, from invasive ductal carcinoma of the breast by hematoxelin and eosin stain (Fig. 1). Malignant cells and high level of mitotic division were observed.

Table (1). RT-PCR primer pairs used in the co- amplification of c-erbB-4 and B-actin RNAs according to (Suo et al., 2002).

Primer Name	Nucleotide sequence 5`-3`	Expected size bp
erbB4-P1	CTC TGG TGG TCT TCC TTC TAC C	**232**
ErbB4-p2	TGA TAG TAG GCA GCA TTG CC	
B-actin-P1	CTT TGA TTG CAC ATT GTT GT	**160**
B-actin -P2	GAA AGC AAT GCT ATC ACC TC	

Table 2. Correlation between Cerb-4 amplification by RT-PCR and estrogen, progestron receptors.

Immuno parameters		Cerb4- RT-PCR					P value
		+ve		-ve		Total	
		No	%	No	%		
ER	+ve	21	65	11	35	32	(p<0.0035) **
	-ve	12	66	6	34	18	
PR	+ve	19	73	7	27	26	(p<0.0001)***
	-ve	14	58	10	42	24	

Correlation between Cerb-4 amplification by RT-PCR and estrogen, progesterone receptors.

Table (2) shows direct statically significant association was detected between Cerb-4 amplification by RT-PCR (Fig.2) and estrogen receptors (ER) expression by immunohistochemistry. 65 % (21/32) of Cerb-4 positive cases were ER positive, While 35 % (11/32) of negative Cerb-4 cases were ER positive. Direct statically significant association was detected between Cerb-4 and progesterone receptors (PR) expression by

immunohistochemistry, 73% (19/26) of Cerb-4 positive cases were PR positive, while 27% (7/26) of negative Cerb-4 cases were PR positive. The material of this work comprised tumor tissue obtained from 50 diagnosed female breast cancer patients presented to the surgical Department, NCI, and Cairo university.during period from 2008 to 2011.Their age ranged from 27 to 60 with a mean of 44.74 ± 1.432, median of 44 years. They underwent surgery either in the form of conservative wide local excision and axillary lymph node dissection or modified radical mastectomy. In this work C-erbB-4 amplification wase determined in 50 newly diagnosed female breast cancer patients with invasive duct carcinoma, trying to correlate such markers with prognostic markers. The mean age of female patients reported in this study was 44.74 years. This was in agreement with results reported by other studies on Egyptian females cancer patients, as those described by El Bolkainy et al., (2010) reported that a mean age of 46.9 years. Western studies reported a mean age of 57 years by Henerson, (2008). However these figures are about 10 years lower than those mentioned by researchers in western countries. Gasparini, (2009), and Chaprin et al., (2009) reported that a mean age of 56.60 and 56.7 years respectively among breast cancer patients. As shown in the results; all cancer cases were IDC the most common histopathological type of cancer breast as it represents 70% of all breast cancer in Western countries and 85.02% in Egypt NCI series (Mokhtar et al., 2007). In the present study, malignant breast tissues invasive duct carcinoma by Hematoxelin and Eosin stain showed groups and clusters of malignant ductal cells, of highly anaplasia and mitosis. This was in agreement with El-Bolkainy et al., (2010). In this study, the incidence of c-erbB-4 amplification was 54% (27/50). Our results were in accordance with Srinivasan, (2009) who studied the prevalence and sites of amplification of c-erbB-4 in 178 human breast cancers cases, which was 49%. Higher incidence was reported by Suo et al., (2009) who studied c-erbB-4 amplification in 100 IDC pateints, it was 82%. This could be explained by the fact that our patients present usually late with big sized tumor and thus higher tumor load. Also this aggressiveness

could be attributed to a biologically different disease. A direct significant association between c-erbB4, and PR and ER status, this was in accordance with Powlawski, (2009), and Suo et al., (2009). So c-erbB-4 amplification could be considered as one of the favorable prognostic markers in cancer breast being directly associated with ER and PR status.

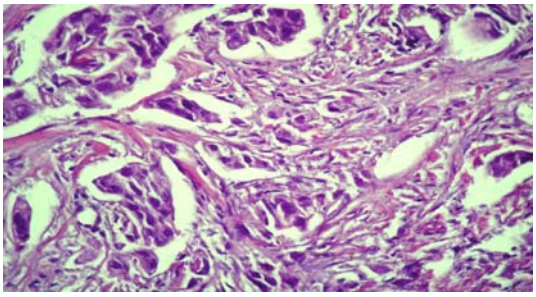

Fig. (1). Histology study. (a) A case of IDC by hematoxelin and eosin stain (X 400) showing mitosis.

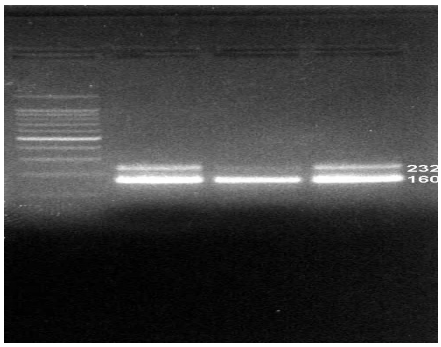

Fig 2. Detection of C-erbB-4 gene amplification, electrophoresis separation of C-erbB-4 (232 bp) and single copy gene B-actin (160 bp) RT- PCR amplified fragments on 2% agarose gel. Lane 2, 4. Positive for C-erbB-4 amplification, lane 3 IDC tumors negative for C-erbB-4 amplification, lane 1: 100 bp molecular weight marker.