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Experimental Study of the Effects of Contraceptive Steroids on Liver Proteins and Nucleic Acids in Mature Female Albino Rats

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Abstract: Effects of administration of a wide range of doses of synthetic estrogens and progestogens on the liver total proteins, albumin and nucleic acids their role for induction of liver growth or liver impairment or as hepato mitogenic agents were studied. The study included 100 mature female Albino rats they were divided into two groups, A and B, group A included 50 rats randomly divided into group A_1: included 20 rats treated with 1.75 ug ethinyl oestradiol in 0.5 mL silicon-oil and group A_2: included 20 rats treated with 7 ug ethinyl oestradiol in 0.5 mL silicon-oil in addition to 10 rats were treated by 0.5 silicon oil and represented the control group (group A_3). Group B: 50 rats randomly divided into group B_1: included 20 rats treated with 15 ug Levonorgestrel in 0.75 mL silicon-oil and group B_2: included 20 rats treated with 200 ug Levonorgestrel in 0.75 mL silicon oil in addition to 10 rats were treated by 0.75 silicon oil and represented the control group (group B_3). Blood samples were used for determination of total proteins, albumin, triglycerides, alkaline phosphatase and γ-glutamyl transferases. Tissues used for the determination of total protein, DNA and RNA contents. The study shows that ethinyl estradiol at 1.75 ug produces significant decrease of the total protein content ($p<0.001$), insignificant increase of the DNA and significant increase of the RNA content ($p<0.001$). Mild fatty degeneration was detected in livers of 3 cases while the rest showed no abnormalities meanwhile ethinyl estradiol at 7 ug produced insignificant increase of the total protein content, significant decrease of the DNA ($p<0.001$) and RNA content ($p<0.001$). Significant decrease of the total protein ($p<0.05$) and the serum albumin ($p<0.001$ while significant increase of the triglyceride ($p<0.001$), γ-glutamyl transferase ($p<0.001$) and alkaline phosphatase ($p<0.05$). Meanwhile, most of the homogenates showed fatty degeneration and cloudy swelling. Levonorgestrel at 15 ug produced significant increase of the mean total protein content ($p<0.01$) and the DNA content but insignificant increase of the RNA content. Meanwhile, insignificant increase of the total protein and significant decrease of the albumin and triglyceride ($p<0.05$ and 0.001) and significant increase of γ-glutamyl transferase ($p<0.001$) and alkaline phosphatase ($p<0.05$) All the animals showed no histopathological changes. Levonorgestrel at 200 ug produced significant increase of total protein ($p<0.001$), insignificant increase DNA content and significant increase RNA content ($p<0.001$). Fatty degeneration and oedema were detected. Significant increase of the total serum proteins ($p<0.001$) but insignificant increase of serum albumin. Significant decrease of triglycerides ($p<0.01$) but significant increase of γ-glutamyl transferase ($p<0.001$) and alkaline phosphatase ($p<0.01$).

Key words: Ethinyl estradiol, levonorgestrel, liver total proteins, albumin, nucleic acids

INTRODUCTION

Estrogen and progestins have been used by millions of women as effective combined contraceptives. The safety of hormonal contraceptives has been documented by years of follow-up and serious adverse events that may be related to their use are rare in the young population exposed to theseagents. The balance between the benefits and the risks of contraceptivesteroids is generally positive in particular when comparing to the risks of pregnancy and especially in women with risk factors (Sitruk-Ware and Nath, 2011).

The metabolic changes induced by the synthetic steroids used in contraception such as lipoprotein changes, insulin response to glucose and coagulation factors have been considered as potential markers of cardiovascular and venous risk. Observations of these effects have led to modifications of the composition of

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hormonal contraceptive in order to minimize these changes and hence potentially decrease the risks. The synthetic estrogen Ethinyl-Estradiol (EE) exerts a stronger effect than natural Estradiol (E2) on hepatic metabolism including estrogen-dependent markers such as liver proteins. This stronger hepatic impact of EE has been related to its 17α-ethinyl group which prevents the inactivation of the molecule and results in a more pronounced hepatic effect of EE as compared to estradiol. Due to its strong activity, administering EE via a non-oral route does not prevent its impact on liver proteins. In order to circumvent the metabolic changes induced by EE, newer products using more natural compounds such as Estradiol (E2) and Estradiol Valerate (E2V) have been introduced (Sitruk-Ware and Nath, 2011).

The synthetic progestins used for contraception are structurally related either to testosterone or to progesterone. Several new progestins have been designed to bind more specifically to the progesterone receptor and to minimize side-effects related to androgenic, estrogenic or glucocorticoid receptor interactions. Risks and benefits of the newer progestins used in contraception depend upon the type of molecular structure, the type and dose of estrogen associated in a combination and the route of administration. The lower metabolic impact of estradiol-based combinations may result in an improved safety profile (Sitruk-Ware and Nath, 2011).

The use of contraceptive patch does not exert a negative effect on carbohydrate metabolism, lipid profile and liverfunction test and blood coagulogram. Total cholesterol, triglyceride and HDL were significantly increased whereas LDL was slightly decreased. The ratio of total cholesterol/HDL and LDL/HDL significantly decreased when applying the patch. After discontinued use of contraceptive patch, the women whose blood tests present hypercholesterolemia during patch use showed a continuous decrease in blood results of total cholesterol level over 3 months. Moreover, mean fasting glucose, SGOT, SGPT and alkaline phosphate were decreased (Kiriwat and Petyim, 2010).

New options for hormonal replacement therapy include non-oral, low-dose sustained delivery of progestins in different formulations. Since, the Women's Health Initiative study, prescription trends were observed selecting progesterone over other progestins or delivering the progestin parentrally, in order to reach low systemic levels of the steroid. Such modalities include progesterone vaginal rings or gels and intra-uterine systems. When given via the transdermal and intranasal routes, the hormones are delivered systemically although the first liver impact is by-passed. Of the progestins selected for delivery of low doses only very active molecules can be used. Although, parental administration of progesterone or low-dose progestins associated with fewer side-effects than oral therapy, the long-term safety of the newer systems warrants further evaluation (Nath and Sitruk-Ware, 2009).

Low dose oral contraceptive produced significant increase of Cholesterol level. No statistically significant differences were observed in LDL and TG concentrations, glycaemia and hepatic function parameters after 12 cycles of the therapy so new low dose oral contraceptive containing 20 μg ethinyl estradiol and 3.0 mg drospirenone does not induce adverse changes in lipids parameters and hepatic function (Szlendak-Sauer *et al*., 2009).

Hepatocellular Carcinomas (HCC), 15-20% occurs in the non-cirrhoticliver. HCC consistently occurring with consumption of oral contraceptives/anabolicsteroids, the carcinogenic effect is mediated by a long-standing alteration of the hepatocellular metabolism that is of low toxic effect and does not lead to cell death but is nevertheless carcinogenic. In these cases, the initial formation of hepatocellular adenomas that subsequently transform into HCC is a common finding (adenoma-carcinoma sequence) (Evert and Dombrowski, 2008). Liver tumor formation in rats treated with oral contraceptive steroids for long periods has been associated with the tumor promoting potential of these agents. These studies suggest that induction of liver growth may be a property relevant for the tumor promoting activity of estrogens; in contrast, induction of hepatic monooxygenases does not appear to be necessary for liver tumor promotion in the rat (Ochs *et al*., 1986).

Aim of the research: Evaluation of the effects of ethinyl estradiol and Levonorgestrel on physiological and high doses on liver proteins and nucleic acids, their role for induction of liver growth or liver impairment or as hepato mitogenic agents.

MATERIALS AND METHODS

The present study was carried out on 100 mature female Albino rats of an average weight 200 g. These rats were kindly provided by the animal house of the faculty of medicine, Assuit University and were fed normal diet. These animals were randomly divided into 2 groups, group A included 50 rats and subdivided randomly into two groups, group A_1 included 20 rats and represented the group treated with the physiological dose of ethinyl oestradiol (1.75 ug) dissolved in 0.5 mL silicon oil and intrapertoneally injected as single dose. Group A_2

comprised 20 rats and represented the group treated with the high dose of ethinyl oestradiol (7 ug) dissolved in 0.5 mL silicon oil and intrapertoneally injected as single dose. The last 10 rats of this group were treated only with intraperitoneal injection of 0.5 mL silicon oil and were used as control.

Group B comprized 50 rats and represented the group of animals treated with the progestogens Levonorgestrel. They were randomly subdivided into the following subgroups, group B_1 included 20 rats that were by intraperitoneal injection of the physiological dose of Levonorgestrel (15 ug) dissolved in 0.75 mL silicon oil as single dose. Group B_2 included 20 rats that were by intraperitoneal injection of the high dose of Levonorgestrel (200 ug) dissolved in 0.75 mL silicon oil as single dose. The last 10 rats of this group were received no progesterone injection but treated only with only intraperitoneal injection of 0.75 mL silicon oil and were used as control.

All rats of the various groups described were kept for 7 days on the same normal diet officially provided by the animal house of the faculty of medicine from the day of injection, after which each was anaesthetized by ether anesthesia and blood samples were withdrawn from the heart by catheterization. Thereafter, the animals were sacrificed and the livers were dissected and weighed. The liver was then cut into two parts. The first part was re-weighed, chilled and was either used fresh or kept frozen for assessment of the parameters of the study that were carried out on tissues. The other part was preserved in formaline and used for the study of the pathological changes occurred in these tissues.

The blood samples were allowed to clot and sera were separated and kept deep-frozen at -20°C for the determinations of total proteins, albumin, triglycerides, alkaline phosphatase and γ-glutamyl transferases.

Tissues used for the determination of the total protein, DNA and RNA contents were these freshly obtained after killing of animals. Serial sections of 4 μm were done. Solids were stained with haematoxylin and eosin. Periodic acid Schiff's stain was performed for liver specimens. The DNA-content was estimated by the method of Parzefall *et al.* (1991). The RNA-content was estimated by Mukundan and Bamji (1976). Total protein content was estimated by Sitruk-Ware and Nath (2011). Serum parameters were determined by using the following methods: total proteins by method of Mukundan and Bamji (1976), albumin by the method of Doumas and Biggs (1972), Triglycerides by the enzymatic method (Bucolo and David, 1973), alkaline phosphatase by the method of Kind and King (1954) and γ-glutamyl transferase by the method of Szasz (1969).

RESULTS

The results of the administration of various doses of the steroids on the homogenates of tissues of liver and blood sera showed that ethinyl oestradiol at 1.75 ug produces significant decrease of the mean total protein content ($p<0.001$) (Table 1, Fig. 1a), insignificant increase

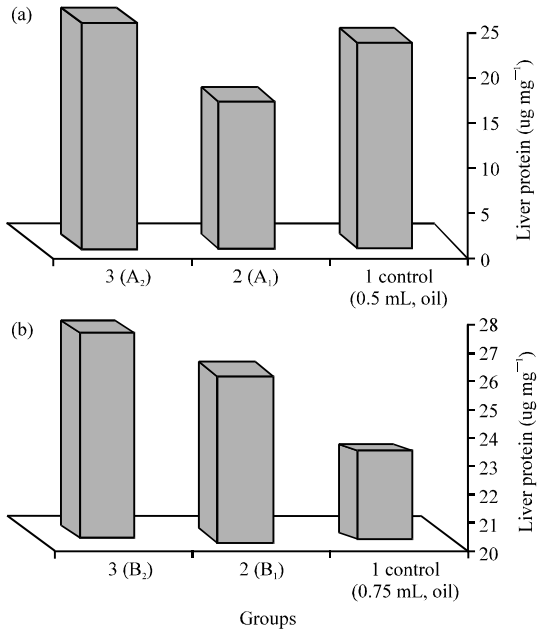

Fig. 1: Liver protein ug/mg wet tissue of the 2 groups of rats, control and those treated with contraceptive steroids; a) Liver total protein ug/mg in group A (treated with ethinyl estradiol). 1: control, 2: group A_1 and 3: group A_2; b) Liver total protein ug/mg wet tissue in group B (treated with Levonorgestrel) 1: control, 2: group B_1 and 3: group B_2

Table 1: Liver total protein ug/mL wet tissue of the various groups of female rats (those treated with different doses of various contraceptive steroids and control)

	Ethinyl Estradiol (EE)			Levonorgestrel (Norgs)		
Statistical analysis	Control 0.5 mL oil	1.75 ug EE	7 ug EE	Control 0.75 ug	15 ug Norgs	200 ug Norgs
Mean±SD	22.57±3.21	16.09±5.934	24.81±5.15	22.86±0.71	25.74±3.46	27.3±3.39
SE	1.02	1.88	1.63	0.22	1.10	1.07
p-value		Sig. decrease $p<0.001$	Insignificant increase $p = 1.165$		Significant increase $p<0.01$	Significant increase $p<0.001$

Table 2: Liver DNA ug/mg wet tissue of the various groups of female rats, control and those treated with different doses of various contraceptive steroids

	Ethinyl Estradiol (EE)			Levonorgestrel (Norgs)		
Statistical analysis	Control 0.5 mL oil	1.75 ug EE	7 ug EE	Control 0.75 ug	15 ug Norgs	200 ug Norgs
Mean±SD	1.10±0.12	1.11±0.294	0.86±0.10	1.07±0.194	1.30±0.15	1.13±0.17
SE	0.04	0.093	0.03	0.062	0.05	0.06
p-value		Insignificant increase p = 0.0988	Significant decrease p<0.001		Significant increase p<0.001	Insignificant increase p = 0.897

Table 3: Liver RNA ug/mg wet tissue of the various groups of female rats, (Those treated with different doses of various contraceptive steroids and controls

	Ethinyl Estradiol (EE)			Levonorgestrel (Norgs)		
Statistical analysis	Control 0.5 mL oil	1.75 ug EE	7 ug EE	Control 0.75 ug	15 ug Norgs	200 ug Norgs
Mean±SD	3.98±0.37	5.2±0.294	2.8±0.32	4.13±0.27	4.3±0.44	4.67±0.14
SE	0.12	0.093	01	0.09	0.44	0.04
p-value		Significant increase p<0.001	Significant decrease p<0.001		Insignificant increase p = 1.1.54	Significant increase p<0.001

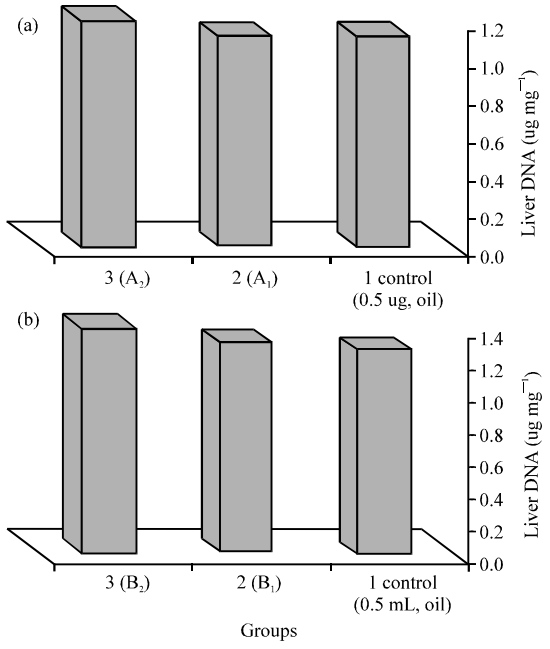

Fig. 2: Liver DNA ug/mg wet tissue of the 2 groups of rats, control and those treated with contraceptive steroids; a) liver DNA ug/mg in group A (treated with ethinyl estradiol). 1: control, 2: group A_1 and 3: group A_2; b) liver DNA ug/mg wet tissue in group B (treated with Levonorgestrel); 1: control, 2: group B_1 and 3: group B_2

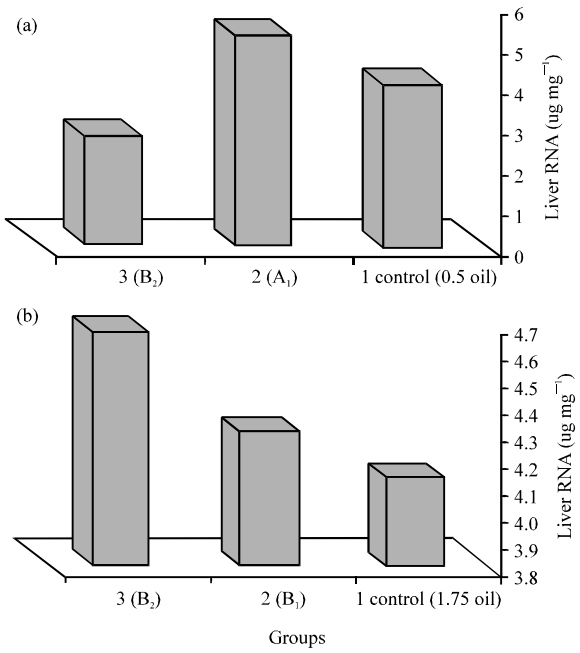

Fig. 3: Liver RNA ug/mg wet tissue of the 2 groups of rats, control and those treated with contraceptive steroids. a) Liver RNAug/mg in group A (treated with ethinyl oestradiol). 1: control, 2: group A_1 and 3: group A_2; b) liver RNA ug/mg wet tissue in group B (treated with Levonorgestrel); 1: control, 2: group B_1 and 3: group B_2

of the mean DNA (Table 2, Fig. 2a) and significant increase of the mean RNA content ($p<0.001$) (Table 3, Fig. 3a). Mild fatty degeneration was detected in livers of 3 cases while the rest showed no abnormalities (Fig. 4) meanwhile ethinyl oestradiol at 7 ug produced insignificant increase of the mean total protein content (Table 1, Fig. 1a), significant decrease of the mean DNA ($p<0.001$) (Table 2, Fig. 2a) and mean RNA content ($p<0.001$) (Table 3, Fig. 3a). Significant decrease of the mean total protein ($p<0.05$) and the mean serum albumin ($p<0.001$), increase of the mean total serum globulin levels which is significant with 1.75 ug, significant decrease of the means of A/G ratio while significant increase of the mean triglyceride ($p<0.001$), γ-glutamyl transferase ($p<0.001$) and the mean alkaline phosphatase ($p<0.05$) (Table 4). Meanwhile, most of the homogenates showed moderate degree of fatty degeneration and cloudy swelling was detected in livers of 19 cases out of 20.

Table 4: Changes of mean serum totalproteins, albumin, triglycerides, γ-glutamyl transferase and alkaline phos phatase concentrations following use of contraceptive steroids as compared to normal control

	Ethinyl Estradiol (EE)			Levonorgestrel (Norgs)		
Parameters	Control 0.5 mL oil	1.75 ug EE	7 ug EE	Control 0.75 ug	15 ug Norgs	200 ug Norgs
Total proteins	7.54±0.6220	6.94±0.711 Significant decrease p<0.05	6.45±1.331 Significant decrease p<0.01	5.61±0.640	6.12±0.741 Insignificant increase, p=1.6	6.44±0.51 Significant increase p<0.001
Albumin	4.18±0.4100	3.11±0.314 Significant decrease p<0.05	2.96±0.67 Significant decrease p<0.001	3.42±0.510	3.06±0.21 Significant decrease, p<0.05	3.74±0.39 Insignificant increase, p = 1.6
Globulin (g dL^{-1})	3.36±0.4530	3.83±0.462	3.49±0.69	2.19±0.300	3.06±0.67	2.70±0.37
A/G ratios	1.264±0.205	0.82±0.08	0.85±0.074	1.59±0.300	1.05±025	1.13±1.41
Triglycerides (mg dL^{-1})	61.85±14.680	169.86±65.42 Significant increase p<0.001	133.19±46.10 Significant increase p<0.001	67.28±25.34	28.6±20.37 Significant decrease, p<0.001	43.98±10.29 Significant decrease, p<0.01
γ-glutamyl	40.58±14.560	187.61±76.1 Significant increase p<0.001	144.26±128.03 Significant increase p<0.01	46.52±13.32	95.53±36.38 Significant increase, p<0.001	80.88±24.88 Significant increase,p<0.001
Alkaline phosphatase	130.57±12.730	173.60±54.91 Significant increase, p<0.05	384.74±166.50 Significant increase, p<0.001	127.97±15.11	164.85±39.96 Significant increase, p<0.05	168.30±39.84 Significant increase,p<0.01

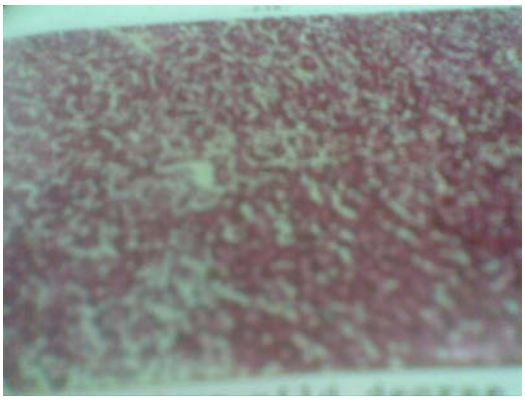

Fig. 4: Liver specimen shows mild degree of fatty degeneration in some cells among the majority of glycogen laden cells. PAS stain

Fig. 5: Liver specimen shows moderate degree of fatty degeneration. H&E stain

Increased glycogen content of liver cells was detected in liver of 10 cases as shown by PAS stain

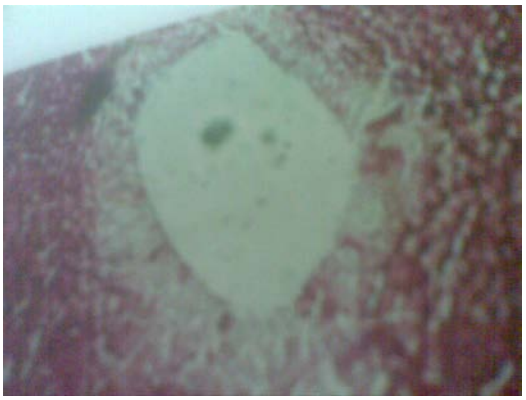

Fig. 6: Liver specimen shows severe degree of central vein dilatation and severe increase in the glycogen contents of the cells. PAS stain

(Fig. 5 and 6). Levonorgestrel at 15 mg produced significant increase of the mean total protein content ($p<0.01$) (Table 1, Fig. 1b) and the mean DNA content (Table 2, Fig. 2b) but insignificant increase of the mean RNA content (Table 3, Fig. 2b) meanwhile insignificant increase of the total protein and significant decrease of the mean albumin and triglyceride ($p<0.05$ and 0.001) and significant increase of the mean γ-glutamyl transferase ($p<0.001$) and alkaline phosphatase ($p<0.05$) (Table 4). All the animals showed no histopathological changes except one which showed cloudy swelling. Levonorgestrel at 200 ug produced significant increase of the mean total protein ($p<0.001$) (Table 1, Fig. 1b), insignificant increase of the mean DNA content (Table 2, Fig. 2b) and significant increase of the mean RNA content ($p<0.001$) (Table 3, Fig. 2b). Fatty degeneration and oedema were detected (Fig. 7). Significant increase of the mean total proteins ($p<0.001$) but insignificant increase of the mean

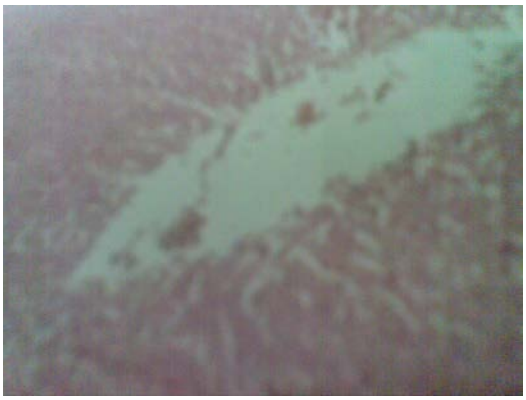

Fig. 7: Liver specimen shows cellular edema and central vein dilatation. H&E stain

albumin. increase of the mean total serum globulin levels but significant decrease of the means of A/G ratio, Significant decrease of the mean triglycerides ($p<0.01$) but significant increase of γ-glutamyl transferase ($p<0.001$) and the mean alkaline phosphatase ($p<0.01$) (Table 4).

DISCUSSION

The present study investigated the effects of the contraceptive steroids, ethinyl oestradiol and Levonorgestrel on liver proteins and nucleic acids and their role in induction of liver growth or liver impairment. These steroids were administered to adult female Albino rats by the intraperitoneal route as single dose oral route was excluded in order to exclude the problem of absorption and the first pass effect.

Ethinyl oestradiol at 1.75 ug produces significant decrease of the total protein content, insignificant increase of DNA and significant increase of RNA content. Mild fatty degeneration was detected in livers of 3 cases while the rest showed no abnormalities. As RNA concentration is usually considered as an index of protein synthesis and growth in bacteria and mammalian tissues, the increased RNA content of liver homogenate is indicative of protein synthesis. In addition, the concentration of protein in any tissue reflects an equilibrium between the rates of biosynthesis and catabolism Therefore, increased RNA content and decrease protein content could be interpretive that biosynthesis of protein does not keep pace with utilization (Parzefall *et al*., 1991; Nemoto and Sakurai, 1995).

Histopathological findings of liver tissue showed mild degree of fatty degeneration in three rats such a finding can support that the utilization of the synthesized protein exceeds the supply of the apolipoprotein of VLDL for the secretion of the newly synthesized triglycerole induced by estrogen, the mechanism responsible for these findings could be resorted to the suggestion that the dose used may not be sufficient to produce maximal translocation of cytosol estrogen receptor to the nucleus (Aten and Eisnefeld, 1982).

Ethinyl oestradiol at 7 ug produced insignificant increase of the total protein content, significant decrease of DNA and RNA content. Histopathological findings showed the presence of moderate degree of fatty degeneration and increased glycogen content. These findings are expected on the assumption that such a dose of the drug can promote hepatotoxic effect, the existence of which seems to be dose dependant however, cloudy swelling could be attributed to disturbance of protein metabolism due to injurious agents and immobilization of fluid from the surrounding lymph by osmosis leading to swelling of the cells and is associated with softening or loosening of the cement substance between cells. In addition the cells exhibit granules that represent coagulation or precipitation of protoplasmic proteins and changes in the cytoreticulum, all these data are indicative of toxic degeneration of liver cells and induction of liver growth may be a property relevant for the tumor promoting activity of estrogens; in contrast, induction of hepatic monooxygenases does not appear to be necessary for liver tumor promotion in the rat (Och *et al*., 1986).

Levonorgestrel at 15 mg produced significant increase of the total protein content and DNA content but insignificant increase of RNA content, these results are indicative of the absence of increased protein synthesis or growth of cells. It could be attributed to increase in cell packing, decrease cell size but without any effect on cell number. The absence of histopathological findings is supportive that such a dose of Levonorgestrel has no stimulatory or inhibitory effects on liver growth or structure (Elouni *et al*., 2010).

Levonorgestrel at 200 ug produced significant increase of the total protein, insignificant increase of DNA content and significant increase of the RNA content. The increased total protein and RNA content without change in DNA content could be explained by increased functional performance without proliferation or growth of cells. Fatty degeneration and oedema were detected which is an indication that such a dose is capable of induction of a hepatotoxic effect. It seems essential to emphasize that such a dose used is about 1 mg kg^{-1} which is much higher than that used for contraceptive use (Huang *et al*., 2010). The data thus obtained in the present study can

show that higher doses of the Levonorgestrel can have a hepatotoxic effect on liver cells and that the severity is dose dependant.

Administration of ethinyl oestradiol results in significant decrease of the total protein and serum albumin, increase of the total serum globulin levels which is significant with 1.75 ug, significant decrease of A/G ratio while significant increase of the triglyceride, γ-glutamyl transferase and alkaline phosphatase meanwhile administration of Levonorgestrel results in insignificant increase of the total protein and significant decrease of the serum albumin and triglyceride ($p<0.05$ and 0.001) and significant increase of γ-glutamyl transferase ($p<0.001$) and alkaline phosphatase with the dose of 15 ug. Significant increase of the total proteins but insignificant increase of serum albumin. Increase of total serum globulin levels but significant decrease of A/G ratio, significant decrease of triglycerides but significant increase of γ-glutamyl transferase and alkaline phosphatase with the dose of 200 ug.

The results obtained from the present study indicate that the administration of contraceptive steroids results in a decrease in the serum total proteins and albumin concentration, the extent of which seems to be dose dependant and appears to be more marked with estrogens rather than progestogens. Levonorgestrel appears to have the same lowering effect on albumin but its effect on total proteins appears to be the opposite.The same results have been reported by Sitruk-Ware and Nath (2011) and El-Sharaky *et al.* (2010). Alternatively, increased hepatic synthesis of triglycerides is attributed by other investigators to increased insulin secretion and altered apoptosis due to their effect on lipoprotein lipase.

The finding of elevation of serum γ-glutamyl transferase activity associating the use of contraceptive steroids, irrespective of the type and dose could be considered as indication of a potential effect of these steroids on hepato billiary system as well as hepatocytes function where the extent of rise could reflect the extent of liver cell affection or the billiary system affection, this in turn could be supported by the findings of the histopathological examination of the liver. In this respect, it seems worthy that ethinyl oestradiol is the most potent steroid in its potential toxic effect and that the Levonorgestrel is much less toxic (Alvaro *et al*., 2002).

The administration of ethinyl oestradiol and Levonorgestrel in the various doses including those for the contraceptive use resulted in significant elevation of serum alkaline phosphatase compared to that of normal control, the extent of which seems to be dose-dependant.

In addition, it is apparent that the maximum elevation is associated with the use of estrogen components rather than the progestogenic ones (Rabaglio *et al*., 2010).

CONCLUSION

The use of ethinyl oestradiol as shown by the histopathological findings indicates that ethinyl oestradiol administration is associated with hepatotoxicity whatever the dose used being that for contraceptive use or higher doses. Low dose of estrogen produced protein biosynthesis that does not keep pace with utilization low dose of Levonorgestrel increased cell packing and decreased cell size without affection of the cell number rather than increased protein synthesis and cellular growth meanwhile on high doses they increased protein synthesis, increasing the protein content of the cells for increasing functional performance but without any proliferation or growth of the cells. However, such a dose seems capable of induction of some hepatotoxic effects.

REFERENCES

Alvaro, D., A. Gigliozzi, L. Marucci, G. Alpini and B. Barbaro *et al*., 2002. Corticosteroids modulate the secretory processes of the rat intrahepatic billiary epithelium. Gastroenterology, 122: 1058-1069.

Aten, R.F. and A.J. Eisnefeld, 1982. Oestradiol is less potent than *Ethinyl oestradiol* for *in vivo* translocation of mammalian liver estrogen receptor to the nucleus. Endocrinology, 111: 1292-1298.

Bucolo, G. and H. David, 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., 19: 476-482.

Doumas, B.T and H.G. Biggs, 1972. Determination of Serum Albumin. In: Standard Methods of Clinical Chemistry. Cooper, G.R. (Ed.). Vol. 7. Academic Press, New York, Pages: 175.

El-Sharaky, A.S., A.A. Newairy, N.M. Elguindy and A.A. Elwafa, 2010. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. Food Chem. Toxicol., 48: 3354-3361.

Elouni, B., C. Ben Salem, M. Zamy, N. Ganne, M. Beaugrand, K. Bouraoui and M. Biour, 2010. Cytolytic hepatitis possibly related to levonorgestrel/ethinylestradiol oral contraceptive use: 2 case reports. Ann. Pharmacother., 44: 2035-2037.

Evert, M. and F. Dombrowski, 2008. Hepatocellular carcinoma in the non-cirrhotic liver. Pathologe, 29: 47-52.

Huang, T., Z.Z. Fang and L. Yang, 2010. Strong inhibitory effect of medroxyprogesterone acetate (MPA) on UDP-glucuronosyltransferase (UGT) 2B7 might induce drug-drug interactions. Pharmazie, 65: 919-921.

Kind, P.R.N. and E.J. King, 1954. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino antipyrine. J. Clin. Pathol., 7: 322-326.

Kiriwat, O. and S. Petyim, 2010. The effects of transdermal contraception on lipid profiles, carbohydrate metabolism and coagulogram in Thai women. Gynecol. Endocrinol., 26: 361-365.

Mukundan, M.A. and M.S. Bamji, 1976. Effect of Ovulen-50 (ethynodiol diacetate 1 mg and ethynyloestradiol 0,05 mg) on protein, nucleic acids and nucleases in the rat liver. Int. J. Vitam Nutr. Res., 46: 492-495.

Nath, A. and R. Sitruk-Ware, 2009. Parenteral administration of progestins for hormonal replacement therapy. Eur. J. Contracept Reprod Health Care, 14: 88-96.

Nemoto, N. and J. Sakurai, 1995. Glucocorticoid and sex hormones as activating or modulating factors for expression of Cyp2b-9 and Cyp2b-10 in the mouse liver and hepatocytes. Arch. Biochem. Biophys., 319: 286-292.

Ochs, H., B. Dusterberg, P. Gunzel and R. Schulte-Hermann, 1986. Effect of tumor promoting contraceptive steroids on growth and drug metabolizing enzymes in rat liver. Cancer Res., 46: 1224-1232.

Parzefall, W., E. Erber, R. Sedivy and R. Schulte-Hermann, 1991. Testing for induction of DNA synthesis in human hepatocyte primary cultures by rat liver tumor promoters. Cancer Res., 51: 1143-1147.

Rabaglio, M., B. Ruepp and Soft/Text/Perche Steering Committee, 2010. Death due to liver failure during endocrine therapy for premenopausal breast cancer. Acta Oncol., 49: 874-876.

Sitruk-Ware, R. and A. Nath, 2011. Metabolic effects of contraceptive steroids. Rev. Endocr. Metab. Disord., 12: 63-75.

Szasz, G., 1969. A kinetic photometric method for serum γ-glutamyl transpeptidase. Clin. Chem., 15: 124-136.

Szlendak-Sauer, K., S. Radowicki and K. Skorzewska, 2009. The impact of a new low dose oral contraceptive containing drospirenone on lipid profile, carbohydrate metabolism and hepatic function. Ginekol. Pol., 80: 99-102.