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The Protective Effects of Selected Mixed Herbal Extracts on Weight, Serum and Liver Tissue in Rats Before and after Exposure to Aflatoxin B_1

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Abstract: Herbs are present in the human diet act as cancer chemopreventive agents. This study's data has been obtained in two ways: firstly, mixed herbal extracts taken before 10 days; secondly, the same mixed herbal extracts taken before 20 days to act as a protective agent on weight, serum and liver tissue in rats, after exposure to aflatoxin B_1 (AFB_1) 0.1 ml/100gm, administered intraperitoneal, in male Wister Albino rats for a period of 10 days to induce liver cancer for both cases. These extracts include, Green tea and Sage (leaves), Fenugreek and Caraway (seeds), Galangal and Ginger (rhizomes), Frankincense and Myrrh (resins), Cinnamon and Cinchona (barks). The animals, were measured for body weight and blood was tested for some key enzymes such as: aseparate aminotransferase (AST), alanine aminotransferase (ALT), gamma- glutamyl transferase (GGT) and other non-enzymatic biochemical parameters including bilirubin, urea, uric acid, creatinine, cholesterol, triacylglycerols and glucose at 10, 20 and 30 days of the experiment. At the end of the experiment (30 days) the rats were killed and were livers removed to be weighed and divided into 2 parts. They were examined, firstly, to determine the content of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and total proteins and, secondly, used for histological examination. The results of this study demonstrated that the group which had taken the mixed herbal extracts before 20 days aflatoxin B_1 exposure had results in improvement in the liver cancer tissues compared to the group that had taken the same mixed herbal extracts before 10 days.

Key words: Aflatoxin B_1 · Liver cancer · Herbal extracts · Biochemical Parameters · Histological examination

INTRODUCTION

In some countries, aflatoxin, the environmental toxin obtained from food spoilage moulds and in particular from mouldy ground nuts, has been strongly associated with the development of tumor in young people, who do not necessarily progress to cirrhosis [1].

Mixed Herbal Extracts in this Study Include: Green tea leaves (*Camellia sinensis*) from the botanical family of *Theacea*, which has demonstrated to display cancer chemopreventive affects in different systems due to its striking inhibition of diverse cellular events related to cancer development [2]; Sage leaves (*Salvia officinalis*) from the botanical family of *Labiatae*, which has potent suppressive activities against tumor promotion in rats and thus could be an effective, chemopreventive agent against skin cancer [3]; Fenugreek seeds (*Trigonella foenum gracum*) from the botanical family of *Legomenaca*, which has been reported to have medicinal use including anticancer properties and antioxidant effects [4]; Caraway seeds (*Carum carvi*) from the botanical family of *Umbelliferae*, which had an antioxidant profile and inhibited tumorigenesis [5]; Galangal rhizomes (*Alpinia officinarum*) from the botanical family of *Zingiberaceae*, which has effect of chemoprevention and anti-prompting activity in tumor [6]; Ginger rhizomes (*Zingiber officinale*) from the botanical family of *Zingiberaceae*, which has shown to be anti-tumor and antioxidative and anti-inflammatory effects [7]; Frankincense resins (*Gum olibanum*) from the botanical family of *Burseraceae*, used as anti-proliferative toward a variety of malignant cells and antioxidant [8]; Myrrh resins (*Commiphora myrrha*) from the botanical family of *Bureseraceae*; which shown to be anti-inflammatory and anti-tumor [9]; Cinnamon barks (*Cinnamomum zeylanicum*) from the botanical family of *Lauraceae*, has shown antioxidant and effectiveness against aflatoxin [10]; Cinchona barks (*Cinchona officinalis*) from the botanical family of *Rubiaceae*; can inhibits microsomal enzymes, thereby inhibiting the bioactivation of toxic compounds and acting as antioxidant, anti-proliferative to a potential hepato-protective and showing tumor-static action [11].

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The aim of this study is to find out the change between increase and decrease in the body weight and some biochemical changes in rats caused by Aflatoxin B_1, on the blood and liver tissues of male Wister Albino Rats. Additionally, the changes and effects of herbal extracts mixed together for a protective effect for 10 and 20 days for the treatment of liver cancer will be studied. Finally, study the histological changes of liver tissue and cells caused by the treatments will be studied and to record the degree of cancer trauma and prognosis of the treatments.

MATERIALS AND METHODS

Subject: In this study, eighty Male Wister Albino Rats, weighing 70-100 gm were maintained in clean cages. The rats were fed with commercial pelleted diet obtained from King Fahad Medical Research Center in Jeddah. The duration of the experiment was 30 days. The rats were divided into 4 groups, each group containing 20 rats. The first group (A) acted as non-tumor bearing control. The second group (B) consisted of rats which were injected i/p with 20 µl (0.1 ml/100gm) B.W aflatoxin B_1 [12] one time from the first day and left for 30 days which served as tumor bearing control. Third group (C) was pre-treated by herbal extracts 10 days prior to the aflatoxin B_1 injection (i.p) and continued to be treated till the 30th day. The fourth group (D) was pre-treated by herbal extracts for 20 days prior to the aflatoxin B_1 injection (i.p) and continued to be treated till the 30th day.

Methods: After 10, 20 and 30 days of the experiment, the rats were weighed and then anesthetized with ether and blood was collected from the heart and selected enzymatic biochemical parameters were determined, including enzymes such as aspartate aminotransferase (AST) [13], alanine aminotransferase (ALT) [14] and gama-glutamyl aminotransferase (GGT) [15] and non-enzymatic biochemical parameters including bilirubin [16], urea [17], uric acid [18], creatinine [19], cholesterol [20], triacylglycerols [21] and glucose [22] which were measured by Dimension DADE BEHRING Company, Germany). In addition, body weights were taken in 10, 20 and 30 days. At the end, the rats were killed and the livers were removed to be weighed and divided into 2 parts, first part to determine deoxyribonucleic acid (DNA) [23], ribonucleic acid (RNA) [23] and total proteins [24]. the The second part were put in formalin solution (10 %) and stained by Hematoxylin and Eosin (H & E) to be used for histological examination [25].

Rats in the 3rd and 4rd groups were pre-treated with the mixed herbal extracts, prepared by heating distilled water (400 ml) to 80°C and soaking 20 gm from each herb for 60 min. After cooling to room temperature, the dose was given orally through special drinking bottles daily [26].

Data Analysis: Collected data were calculated by T-test and ANOVA using SPSS program, version 15.

RESULTS

Significant increases in body weight on the 10th, 20th and 30th days and in the liver weight on day 30 when Group (B) was compared with Group (A). In Group (C) there was highly significant increases in the body weight on the 10th and 30th days compared with both Groups (A) and (B) and an increase on day 20 compared with Group (A). Regarding liver weight, there was slight increase on day 30 when compared with group A but a decrease when compared with Group (B). Group (D), shows an increase in body weight on day 10 when compared with Group A, but a decrease was seen when compared with Group B and a decrease on day 20 when compared with both Groups (A) and (B). However, significant increases on day 30 when compared with Group (A), but significant decreases when compared with Group (B). The liver weight decreases on day 30 when compared with both Groups (A) and (B) (Table 1).

Additionally, some key enzymes (AST, ALT and GGT) from Group (B) were compared with Group (A). The results showed a slight change in the activity of AST, a decrease in the activity of ALT and a significant increase in the activity of GGT. In Group (C), there was a decrease in the activity of AST on day 10 and day 30 when compared with both Groups (A) and (B), a decrease on day 20 when compared with Group (A), but an increases was seen when compared with Group (B). In Group (D), there is a decrease in the activity of AST on the 10th day but an increases on the 20th and 30th days when compared with both Groups A and B. In Group (C), there is a significant increases in the activity of ALT on day 10, but this activity decreases on the 20th and 30th days when compared with both Groups (A) and (B). In Group (D), there is significant decrease in the activity of ALT on the 10th days, however, this activity shows significant increases on the 20th day and increases on 30th day when compared with both Groups (A) and (B). In Group C, we see an increase in the activity of GGT on

Table 1: Mean value±SD of body and liver weight in non-tumor bearing control group (A), tumor bearing control group (B), pre-treated by herbal extracts before 10 days group (C) and pre-treated by herbal extracts before 20 days group (D)

Groups Weight	Group (A)	Group (B)	Group (C)	Group (D)
Body weight (10 days) gm	67.7±3.5	84.3±3.2*	100±4.4**	75.3±12.8
Body weight (20 days) gm	114±6.6	137.3±10.2*	118±4.4	100±9.2
Body weight (30 days) gm	100.6±2.1	127.3±6.7*	132.7±3.8**	124±18.2*
Liver weight (3o days) gm	8.2±0.1	9.9±1*	8.6±1.3	6.7±0.5

Data are presented as mean±S. D. ; S.D. = Standard deviation ; *Significant P < 0.05 ; ** Highly significant P < 0.001

Table 2: Mean value±SD of some key enzymes (AST, ALT, GGT) in non-tumor bearing control group (A), tumor bearing control group (B), pre-treated by herbal extracts before 10 days group (C) and pre-treated by herbal extracts before 20 days group (D)

			Group (C)			Group (D)		
Groups Parameters	Group (A)	Group (B)	10 days	20 days	30 days	10 days	20 days	30 days
AST(U / L)	333±251.9	315±172.2	180±35	325.7±38.4	280±52.9	282±28.6	586 ±65.9	356.3± 66.4
ALTU / L))	79.7 ±9.9	72±7.8	48±7.2*	66 ±34.2	59±35.7	29.3 ±9.3*	151 ±58.4*	88.3 ±12.6
GGT(U / L)	12 ±1.7	15±1	14 ±2.6	13±1	13 ±1	11 ±3.6	13 ±1	13 ±0.6

Data are presented as mean±S.D.; S.D. =Standard deviation; * Significant P < 0.05

day 10, 20 and 30 when compared with Group (A), but this activity decreases when compared with Group B. In Group (D), there is a decrease in the activity of GGT on day 10, however, an increases on the 20th and 30th days was seen when compared with Group (A), but a decrease in this activity in the same periods was seen when compared with Group (B) (Table 2).

Non-enzymatic biochemical parameters (bilirubin, urea, uric acid, creatinine, cholesterol, triacylglycerols and glucose) from the Group (B) are compared with Group (A). There is a highly significant increase in bilirubin levels, an increase in urea levels, significant increase in uric acid and a slight increase in creatinine level. There is also a decrease of cholesterol and triacylglycerol level. In addition, an increase in glucose level is also noticeable. Additionally, non-enzymatic biochemical parameters were studied. In Group (C) and (D), there are a very highly significant decreases in the level of bilirubin at all periods of the experiments when compared with both groups (A) and (B). In Group (C), a decrease in the level of urea on day 10 and significant decreases on the 20th and 30th days are detected. In Group (D), an increase in these levels at all period of the experiments is shown when compared with both Groups (A) and (B). In Group (C), there is a decrease in the level of uric acid on day 10 and significant decreases on day 20 and 30 when compared with both Groups (A) and (B). In Group (D), a slightly decreases on day 10 and significant decreases on day 20 and 30 when compared with Group (A) however, an increase on day 10 and significant decreases on day 20 and 30 when compared with Group (B). In Group (C), level of creatinine is the same on day 10 but decreases on day 20 and 30 when compared with both Groups (A) and (B). Additionally, this level increases in Group (D) on day 10 and stayed the same on day 20 and 30 when compared with both Groups (A) and (B). In Group (C), there is an increase in the level of cholesterol on day 10 and very highly significant decreases on day 20 but an increases on day 30 is seen when compared with both Groups (A) and (B). In Group (D), an increase in the level of cholesterol on day 10, very highly significant decreases on day 20 and 30 when compared with both Groups (A) and (B). In Group (C), there is a decrease in the level of triacylglycerol on day 10 and 20, very highly significant increase on day 30 when compared with Group (A). In addition, a very highly significant increase on day 30 and increase on day 20 but this level decreases on day 10 when compared with Group (B). In Group (D), there is a decrease in the triacylglycerol level on day 10 and 20 and a significant decrease on day 30 when compared with Group (A), an increase on day 10 and 20 but a significant decrease on day 30 when compared with Group (B). In Group (C), there is a very highly significant increase in the level of glucose on day 10, significant increase on day 30 and increase on day 20 when compared with Group (A) but when compared with Group (B) the same level on day 10, a decrease on day 20, but significant increase on day 30 in the level of glucose were seen. In Group (D), a very highly significant increase on day 10 and 20 and significant increase on day 30 are seen when compared with both Groups A and B (Table 3).

Table 3: Mean value±SD of some non-enzymatic biochemical parameters (bilirubin, urea, uric acid, creatinine, cholesterol, triacylglycerol and glucose) in non-tumor bearing control group (A), tumor bearing control group (B), pre-treated by herbal extracts before 10 days group (C) and pre-treated by herbal extracts before 20 days group (D)

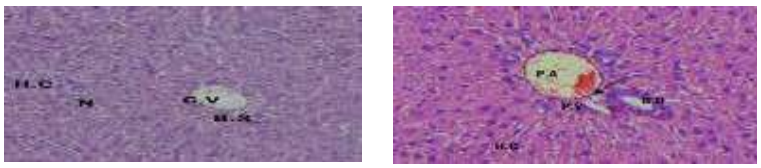
			Group (C)			Group (D)		
Groups Parameters	Group (A)	Group (B)	10 days	20 days	30 days	10 days	20 days	30 days
Bilirubin (mg / dl)	1.2±1	2.2±0.1***	0.2±0.1***	0.03±0.02***	0.13±0.1***	0.7±0.1***	0.2±0.00***	0.3±0.1***
Urea(mg /dl)	27±1.7	28±2.6	23±1	23±1*	20.7±2.1*	29.7±8	29±2.6	29±1
Uric acid(mg / dl)	3.9±0.9	2.3±0.8*	2.5±1.1	1.7±0.6*	2±0.9*	3.5±0.3	1.8±1*	2.1±0.9*
Creatinine(mg / dl)	0.2±1	0.2±0.01	0.2±0.1	0.1±0.1	0.11±0.1	0.3±0.2	0.2±0.1	0.21±0.1
Cholesterol(mg /dl)	94.3±5	82.3±2.1	99.7±37.3	56.7±3.2***	95.7±7.4	86.7±7.5	62.7±3.1***	70.3±2.5***
Triacylglycerol(mg / dl)	73.3±22.3	57±1	55.3±7	60±17.6	160±45.6***	62.3±3.2	68±4	45.7±9*
Glucose(mg / dl)	137±1	171±2***	171±1***	143±13	193±20.1*	185±13.6***	190.7±16.1***	180±22.9*

Data are presented as mean±S. D.; S. D. =Standard deviation; * Significant P < 0.05; *** Very highly significant P < 0.000

Table 4: Mean value±SD for liver content of DNA, RNA and total proteins (gm/100gm) in non-tumor bearing control group (A), tumor bearing control group (B), pre-treated by herbal extracts before 10 days group (C) and pre-treated by herbal extracts before 20 days group (D)

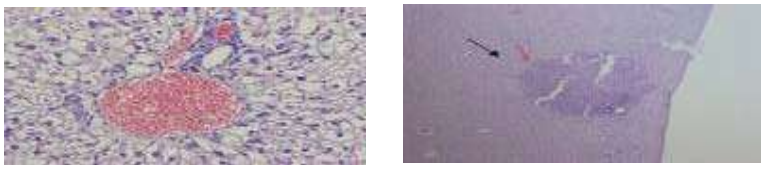
Groups Parameters	Group (A)	Group (B)	Group (C)	Group (D)
RNA (gm/100 gm)	2.1±0.7	1.3±0.1*	2.03±0.1	1.9±0.01
DNA (gm/100 gm)	0.73±0.1	0.5±0.1*	1.3±0.1***	1.4±0.1***
Total protein (gm/100 gm)	6.8±0.7	5.5±1.4	9.1±2.5	9.8±0.8

Data are presented as mean±S.D.; S.D. =Standard deviation; * Significant P < 0.05; *** Very highly significant P < 0.000



(a) (b)

Fig. 1a,b: **a:** A part of liver from group (A) showing hepatic cells (H.C) around the central vein (C.V), nucleus (N), and blood sinusoid (B.S).Hematoxylin & Eosin (H&E) (X 400)
b: A part of liver from group (A) showing portal area (P.A), which is contain portal vein (P.V), bile duct (B.D), inside the endothelial tissue (arrow) and laminal of hepatic cells (H.C). Hematoxyline& Eosin (H&E) (X 400)

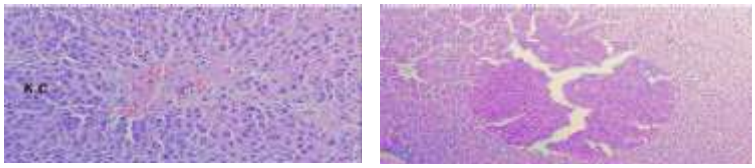


(a) (b)

Fig. 2a,b: **a:** A part of liver from group (B) showing degenerative, necrotic hepatic cells, hemorrhage in the portal area.Hematoxylin & Eosin (H&E) (X 400)
b: A part of liver from group (B) showing hepatoma focci (arrow). Hematoxylin & Eosin (H&E) (X 400)

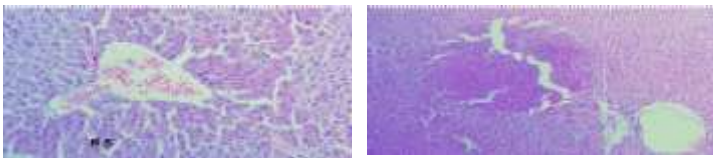
In general, significant decrease in RNA, DNA levels and a decreases in the level of total protein obtained from liver tissue in Group (B) compared with Group (A). In Group (C), there is significant increase in the level of RNA. In Group (D), a very highly significant increase is shown in the level of RNA. In Group (C) and (D), there are very highly significant increases in the level of DNA and an increases in the level of total protein are shown in liver tissue when compared with both Groups (A) and (B) (Table 4).

Histological examination of the liver tissue is shown in (Figure 1 a, b and Figure 2 a, b), When compared



(a) (b)

Fig. 3a,b: **a:** A part of liver from group (C) showing many hepatocytes were binucleated accompanied by granular degeneration and fatty changes activation of Kupffer cells (K.C). Hematoxylin & Eosin (H&E) (X 400)
b: A part of liver from group (C) showing hepatoma foci with patches of coagulative necrosis.Hematoxylin & Eosin (H&E) (X 400)



(a) (b)

Fig. 4a,b: **a:** A part of liver from group (D) showing some hepatocytes arranged in duct like from around central vein and many blood sinusoid (B.S).Hematoxylin & Eosin (H&E) (X 400)
b: A part of liver from group (D) showing hepatoma foci with necrotic patches.Hematoxylin & Eosin (H&E) (X 400)

between Group (A) and (B), they indicate the liver cells were seen without nucleus, degenerative, hepatoma foci as well as a decrease in the number of Kupffer cells.

In addition, histological examination of liver tissue is seen in (Figure 3 a, b and 4 a, b), for Group (C) and (D), respectively. In Group (C), many hepatocytes were binucleated accompanied by granular degeneration and fatty changes and activation of Kupffer cells and hepatoma foci with patches of coagulative necrosis. In Group (D), some hepatocytes arranged in duct like form around the central vein and many blood sinusoid (B.S), hepatoma foci with necrotic patches.

DISCUSSIONS

When comparing Group (B) with the Group (A) the results showed, very highly significant increase for body weight in 10, 20 and 30 days, also similar results was also seen in liver weight in 30 days, these increases in both body and liver weight is due to exposure of Aflatoxin B_1.

Looking at some key enzymes and non-enzymatic biochemical parameters results from exposure to aflatoxin B_1, the results showed a significant increase in the activity of GGT, highly significant increase in bilirubin serum level. Observed through liver tissue, a significant decrease in RNA level was seen. Histological examination showed that liver cells were seen without nucleus, degenerative, hepatoma foci and decreased in the number of kupffer cells. The results may be due to degeneration of GGT from the wall of hepatic cells to the blood, which correlate with the histological results.

There was highly significant increase in bilirubin level following the administration of aflatoxin B_1 due to the degeneration of the heme of hemoglobin in red blood cells [27]. A decrease in triacyglycerols and cholesterol levels conclude that an increase of lipolysis in fat tissue and the metabolic alterations in the liver precede catabolic reactions in peripheral tissues [28].

From the results, a decrease in DNA level due to AFB_1 DNA adduct is shown a short time after the administration which might be due to a decrease in protein synthesis[28] which go along with significant change in hepatic protein metabolism but no significant change in skeletal muscles were seen.

Group (B) had altered hepatic protein in a way similar to the results reported[29] where they found a decrease in the level of total protein in the liver due to AFB_1 DNA adduct which may interrupt the transcription process of RNA causing a decrease in the synthesis of protein. Histology findings showed hepatoma foci, degeneration and necrosis of hepatic cells, cells without nucleus and a decrease in the number of Kupffer cells [30].

When comparing the Group (C) with both group (A) and (B) the results showed, very highly significant increase of body weight in 10, 30 days and slight increases of liver weight in 30 days, may be the protective effect of daily drinks of mixed herbal extracts on body and liver weight grow with normal value when it was taken before and after exposure of AFB_1 and cancer preventive components showed inhibitory effects on tumor-promoting stage [31]. Many components derived from dietary or medicinal plants show antioxidant and anti-inflammatory potential and chronic diseases have been found to possess chemopreventive properties.

Key enzymes and non-enzymatic biochemical parameters studied in Group (C) and compared with both Group (A) and (B) showed significant increases in the activity of ALT in 10 days before exposure to AFB_1. The elevation of serum ALT and AST activities due to AFB_1 dosing were almost completely abolished by the treatment of herbal extracts. Other herbal extracts showed immunostimulatory effect and chemopreventive agents that inhibit carcinogen activation [32].

Very highly significant decrease in bilirubin level in all periods of the experiment are seen, due to the effect of some herbal extracts are antioxidant and scavenging free radical that protect cells from tumor [33]. Additionally, significant decrease in the level of urea and uric acid in 20 and 30 days are noticed, which may be due to some herbal extracts work as that can a promising agent to prevent mesangial cell proliferation [34]. A very highly significant decrease in the level of cholesterol in 20 days is also seen, which some herbal extracts in protection against coronary atherosclerosis at least in men and lipid peroxidation in rats. showed that some herbal extract do not only protects normal cells against genotoxic hazard but also eliminates cancer cells through induction of apoptosis [35]. A very highly significant increase in triacylglycerol levels at 30 days is seen when compared with both Group (A) and (B). A very highly significant increase in the glucose level in 10 days, a significant increase in 30 days compared with Group (A) and a significant increase in 30 days was seen only when compared with Group (B) was noticed. The herbal extracts not affecting glucose levels, which oppose the flavonol action in some herbal extracts which inhibit glucuronide transport in the endoplasmic reticulum, reducing the reactivation of carcinogens, inhibiting glucosidase II, which causes endoplasmic reticulum stress and apoptosis in hepatoma cells and they hinder glucose efflux, which may decrease hepatic glucose production and blood glucose level [2]. A significant increases in the level of RNA and very highly significant increases in DNA are seen when compared with both Group (A) and (B). Histological examination showed, many hepatocytes are binucleated accompanied by granular degeneration and fatty changes activation of Kupffer cells and hepatoma foci with patches of coagulative necrosis. Some herbal extracts causes necrosis, congestion hemorrhage in gastric mucosa. The affect of AFB_1 may be greater than the effect of some herbal extracts or it might be due to low dose of extracts [36].

When comparing Group (D) with both Group (A) and (B), results showed a significant increase in body weight in 30 days when compared with Group (A) but a significant decrease when compared with Group (B) and a decrease in liver weight in 30 days may be the protective effect of herbal extracts on body and liver weight, lead to normal growth [37].

Key enzymes and non-enzymatic biochemical parameters studied in Group (D) compared with both Group (A) and (B) showed significant decrease in the activity of ALT in 10 days, however this activity showed significant increases in 20 days. A very highly significant decrease in the level of bilirubin at all period [37]. Significant decrease in uric acid in 10, 20 and 30 days, showed that some herbal extract do not only protects normal cells against genotoxic hazard but also eliminates cancer cells through induction of apoptosis [35]. A very highly significant decrease in the level of cholesterol in 20 and 30 days is shown and this result agrees with the results of other authors where they found that some herbal extract worked in lower hepatic cholesterol content and suppresses lipid peroxidation via enhancement of hepatic antioxidant enzyme activities [38]. A significant decrease in the level of triacylglycerol in 30 days is noticed. A very highly significant increase in the level of glucose in 10 and 20 days and significant increase in 30 days when compared with both Groups (A) and (B) is seen. A very highly significant increase in RNA and DNA levels were shown in liver tissues samples, which diminish the formation of oxidized metabolites of DNA with an associated lower risk of specific types of cancer. Furthermore, some herbal extract is found to exert inhibitory effects on the viability and DNA synthesis of human promyelocytic leukemia. In addition, it markedly suppresses free radical generation, pro-inflammatory protein production accompanied by apoptosis [39].

Histological examination showed some hepatocytes arranged in duct like from around central vein and many blood sinusoid (B.S) and hepatoma foci with necrotic patches. The study demonstrated that the group who had taken mixed herbal medicinal extracts before 20 days

aflatoxin B_1 exposure had results in improvement in the liver cancer tissues compared to the group who had taken the same mixed herbal medicinal extracts before 10 days.

CONCLUSION

Herbs and spices have been used for generations by humans as food and to treat aliments. Scientific evidence is accumulating that many of these herbs and spices do have medicinal properties. The results of this study demonstrated that the group who had taken mixed herbal medicinal extracts before 20 days had results in improvement but not affect in the liver cancer tissues compared to the group who had taken the same mixed herbal medicinal extracts before 10 days and after exposure to aflatoxin B1 for both groups.

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