International Journal of Pharma and Bio Sciences

ANTIDIABETIC ACTIVITY OF *CASSIA OCCIDENTALIS* Linn. IN STREPTOZOTOCIN-INDUCED DIABETIC RATS: A DOSE DEPENDENT STUDY

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ABSTRACT

Cassia occidentalis Linn. is extensively used in the indigenous and folklore medicine systems to treat several illnesses. However adequate characterization of hypoglycemic activity of *C.occidentalis* has not yet been done. The scientific evaluation of its hypoglycemic activity was, therefore, explored and also compared with the effect of a standard hypoglycemic drug, Glibenclamide. In the present study methanol fraction of *C.occidentalis* leaves (*COLMF)* was tested against streptozotocin-induced diabetic rats. Adult male albino Wistar rats, weighing 150-200g, were randomized into control and experimental groups. Experiment group rats were induced diabetes by a single intraperitoneal injection of streptozotocin (STZ). Treatment with *COLMF* at different doses and times following in normal and diabetic rats significantly reduced the blood glucose level to normal in diabetic rats (99.68±3.57). Hemoglobin, glycosylated hemoglobin, hepatic glycogen, lipid peroxidation, antioxidants enzymes (TBARS, HP, SOD, CAT, GPx VitC,VitE,GSH) and hepatic marker enzymes (ALT, AST,ALP,ACP) were also evaluated in normal and diabetic rats. Oral administration of *COLMF* significantly and dose-dependently normalized the above mentioned parameters near to normal in STZ-diabetic rats ($p<0.05$). Histopathological examination showed that *COLMF* extract protected the pancreatic tissue from STZ-induced damage.

KEYWORDS

Cassia occidentalis, Diabetes mellitus, Glibenclamide, streptozotocin,

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by abnormal metabolism of carbohydrate, proteins, fats resulting from defects in inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action.[1] The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and pre-mature death.[2,3] This illness affects approximately 150 million people worldwide and its incidence rate is expected to double during the next 20 years.[4] The modern drugs, including insulin and other oral

hypoglycemic agents such as biguanides, sulphonylureas, alpha-glycosidase inhibitors, control the blood glucose level as long as they are regularly administered and they may also produce a number of undesirable effect[5]. Hence there is a need to search for newer anti-diabetic agents that have high therapeutic efficacy with minimum side effects[6.] Traditional medicines all over the world have advocated the use of herbs to treat diabetes since time immemorial. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of DM[7, 8].

C.occidentalis is extensively used in the indigenous and folklore medicine systems to treat hepatotoxicity. In unani medicine it is used as an antidote of poisons, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases[9]. It is also an important ingredient of several polyherbal formulations marketed for liver diseases. Its roots, flowers, seeds and leaves have been employed in herbal medicine around the world for a variety of purposes such as laxative, expectorant, analgesic, anti-malarial[10], hepatoprotective[11], relaxant[12], anti-inflammatory[13], and wound healing.[14]

No detailed study has been carried out on the effect of hypoglycemic activity *C.occidentalis*. Hence, the present study was aimed to evaluate the efficacy of *COLMF* on blood glucose, plasma insulin levels, hemoglobin, glycosylated hemoglobin, hepatic glycogen, lipid peroxidation, antioxidants enzymes and hepatic marker in plasma and liver of STZ -diabetic rats.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *C.occidentalis* were collected from the premises of Loyola college Chennai, and it was authenticated by Dr. Sri Ram murthy, department of botany, Andhra Loyola college, Vijayawada. A voucher specimen is deposited. The leaves were shade dried, coarsely powdered and used for extraction. Powdered leaf material (3kg) was soaked sequentially in hexane, chloroform, ethyl acetate and methanol for 72 h, respectively with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary evaporator. The filtrates were air dried to yield 26g of hexane extract, 32g of chloroform extract, 38g of ethyl acetate extract, and 42g of methanol extract.

Liquid–liquid partitioning: Active methanolic crude extract was utilized for liquid–liquid partitioning using increasing polarity. Four fractions were obtained, i.e., hexane (13g), benzene (7g), ethyl acetate (9g) and methanol (15.6g). Amongst these fractions methanol fraction exhibited the most potent diabetic activity and was therefore methanol fraction was used throughout the study.

Preliminary phytochemical analysis: *COLMF* fractions were subjected to qualitative phytochemical investigation for the identification of the phytoconstituents, such as terpenoids, steroid, alkaloids, glycosides, saponins, flavonoids and anthraquinones.[15]

Experimental design: Healthy male Albino rats weighing 150-200g were procured from National institute of nutrition, Hyderabad, India. They were acclimatized to animal house conditions, fed with commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and were provided with clean drinking water *ad libitum.* Twelve hours before the start of the experiment, rats were deprived of food, but given free access to water. All the animal experiments were conducted according to the ethical norms approved by Ministry of social justices and empowerment, government of India and institutional animal ethics committee guidelines (1221/a/08/CPCSEA).

Experimental protocol:
Albino Wistar rats weighing 110-150 g were divided into five groups, each containing six animals. COLMF at a dose of 100 200mg/kg bw dissolved in 1 % carboxymethylcellulose administered orally using an intragastric tube for three weeks to the respective groups

***Chemicals and Biochemical measurements*:** STZ was procured from Sigma chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade. All spectrophotometric measurements were carried out using UV2010 Spec-210 trophotometer (Hitachi, Germany).

An oral glucose tolerance test (OGTT) was performed by the method of Du Vigneaud and Vincent (1925). After overnight fasting, a baseline (0 min) blood sample (0.3 ml) was taken in normal and experimental rats by sinocular puncture. Without delay, a glucose solution (2 g kg−1 bw) was administered to all the experimental rats and *COLMF* (100 and 200 mg/ kg bw) was administered to the respective experimental groups. Five more samples were taken after 1h, 3h and 6 h after glucose administration in potassium oxalate and sodium fluoride containing tubes. Plasma was separated and utilized for glucose estimation.

Fasting plasma glucose was estimated by Glucose-oxidase peroxidase method[16]. Haemoglobin was estimated by cyanmethemoglobin method of Drabkin.[19] Glycosylated hemoglobin HbA1c in the blood was estimated by the method of Sudhakar and Pattabiraman [19], (1981).Plasma Insulin was estimated by Rat insulin RIA Kit and Hepatic Glycogen was extracted and estimated by the method of Leloir and Goldemberg.[20]

Superoxide Dismutase (SOD) was assayed by the method of Kakkar *et al*.[21] Catalase (CAT) was determined by the method of Sinha[22]. And Glutathione Peroxidase (GPx) was measured by the method of Rotruck *et al*.[23] Aascorbic acid in the plasma and tissues was estimated by the method of Roe and Kuether.[24] α–Tocopherol in the plasma and tissues was estimated by the method of Baker *et al.*[25] Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were assayed using commercial kits (Dialab, Austria).

***Acute Toxicity Studies*:** The acute toxicity of the extract was conducted by the method of Lorke.[26] Rats fasted for 12 h were randomly divided in to drug treated 'test' groups and vehicle treated 'control' group, totally making up six groups of six rats per cage. *COLMF* (200, 400, 800, 1200 and 1600 mg/kg b.w) was separately administered orally to the rats in each of the test groups, respectively. Behavioral changes (irritation, restlessness, respiratory distress, abnormal locomotion and catalepsy) were observed over a period of 48 h for sign of acute toxicity. The number of mortality caused by the compound within this period of time was observed.

Experimental induction of diabetes in rats: The animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (60 mg/kg bw) in freshly prepared citrate buffer (0.1M, pH 4.5). STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. Diabetes was developed and stabilized in these STZ-induced rats over a period of 7 days. After 7 days of STZ administration, plasma glucose levels of each rat were determined. The animals with blood glucose above 230 mg/dL were considered to be diabetic and used for the experiment.

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes

containing potassium oxalate sodium fluoride mixture and plasma was separated. The liver and pancreas was removed promptly, and weighed. The tissues were stored at -70^{C} until required. A 20% homogenate was prepared in 50mM phosphate buffer, pH 7.4 and were centrifuged and the supernatant was used immediately for the assays different biochemical parameters.

Statistical analysis: All the data were expressed as Mean ± S.D. Tukey-Kramer multiple comparison test and one way analyses of variance (ANOVA) were performed. A value of $P<0.05$ was considered significant.

RESULTS

Preliminary qualitative chemical analysis of *COLMF* revealed the presence of anthraquinones saponins, terpenoids, glycosides, and flavonoids

Acute toxicity studies:

Oral administration of graded doses of *COLMF* to the normal healthy rats did not show any significant alterations in plasma enzymes like AST, ALT, ACP and ALP between the normal control and *COLMF* treated test groups. Acute toxicity studies revealed the non-toxic nature of the *COLMF* (Fig.1). Behavior of the treated rats also appeared normal. There was no lethality or toxic reaction at any selected dose until the end of the study.

Oral Glucose Tolerance test:

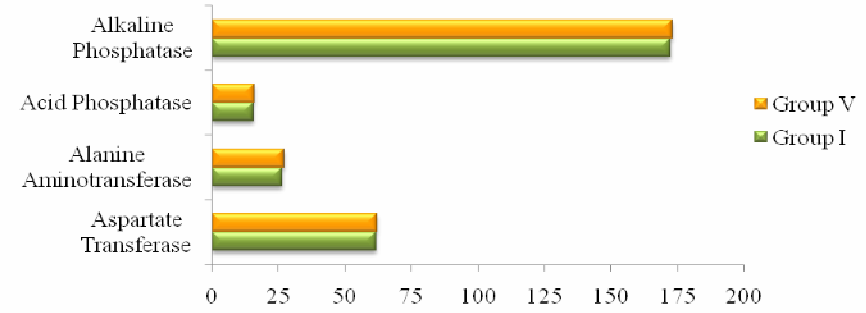
Figure 2 showed the effect of *COLMF* on oral glucose tolerance test in normal and STZ-diabetic rats. In STZ-treated diabetic rats, the peak increase in blood glucose concentration was observed after 1h. Even after 6h the blood glucose concentration in this group remained high. Oral administration of *COLMF* (200 mg/ kg bw) controlled the glucose levels at 1 h (125.86±3.84), 3h (112.18±1.54) and 6 h (99.68±3.57), but no marked change was observed in glucose levels at 100 mg/kg bw.

Effect on Plasma insulin, Hb, HbAlc, hepatic glycogen and blood glucose levels

Table 1 shows the effect of *COLMF* on plasma insulin levels in normal and diabetic rats. In diabetic rat, there was a significant decrease in plasma insulin levels as compared to normal rats. Oral administration of *COLMF* significantly and dose dependently increased the plasma insulin levels to near normal (14.53±1.25) (Table.1).

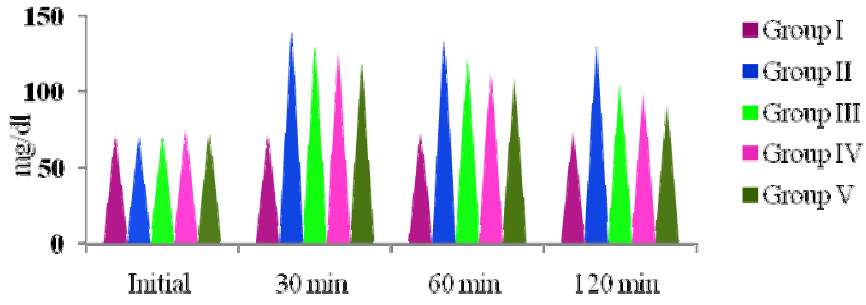
Glycosylated hemoglobin levels were significantly elevated and total hemoglobin, hepatic glycogen were decreased in diabetic rats as compared with normal rats. Oral administration of *COLMF* (200 mg/kg bw) maintained all the parameters in near normal status in diabetic rats ($P<0.001$).

Figure 1: Effect of Methanol crude extract on Serum marker enzymes (µ moles of pyruvate liberated / mg protein /hr) in experimental rats



Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group V is compared with group I.

Figure 2: Effect of *COLMF* on Oral Glucose Tolerance in STZ-diabetic rats



Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group III and IV and V are compared with group II.

Fasting blood glucose was measured after rats were fasted for 12 h on day 45 (last day of treatment) In the diabetic control group, the fasting blood glucose significantly increased, oral administration of *COLMF* dose-dependently and significantly decreased fasting blood glucose level of diabetic rats (P<0.001)

Effect on antioxidants levels: The TBARS, HP, levels in liver, pancreas and plasma of untreated diabetic control rats were significantly higher than those of the untreated normal control rats. When diabetic rats were treated for 45 days, the levels of TBARS, HP were decreased in a dose-dependent manner (P>0.05).

The activities of antioxidant enzymes such as SOD, CAT and GPx (Table 2) and Nonenzymic antioxidants, such as VitC, VitE GSH (Figure 4), were altered in the plasma of untreated diabetic control rats. Treatment with *COLMF* (100, 200 mg/kg bw) dose-dependently and significantly restored the decreased nonenzymic antioxidant levels and antioxidant enzymes activities near normal levels in diabetic rats (P>0.05).

Table 1: Concentration of Hemoglobin (mg/dl), Glycosylated hemoglobin (mg / g of Hb), plasma insulin (IU/L) and hepatic glycogen (mg / 100 g wet tissue) levels in control and control and STZ-diabetic rats

	Group I	Group II	Group III	Group IV	Group V
Hb	14.59 ± 1.42	6.41 ± 0.47	9.19±0.62	0.88 ± 1.40*	11.62 ± 1.26
HbA1c	0.69 ± 0.05	1.17 ± 0.16	0.79±0.17	0.75 ± 0.14*	0.76 ± 0.11
Plasma Insulin	15.68 ± 1.38	6.67 ± 0.59	10.66±1.85	1.86 ± 1.33*	12.90 ± 1.23
Blood glucose	5.76 ± 0.15	17.74 ± 1.25	12.34 ± 1.11	9.13±1.01*	8.36 ± 0.06
Hepatic Glycogen	5.25 ± 0.82	1.79 ± 0.63	3.81±0.83	4.69 ± 0.77*	5.08 ± 0.74

Values are expressed as mean ± S.D (n=6) statistically significant alterations are expressed as $p<0.05$.* Group III and IV and V are compared with group II

Table2: Concentration of antioxidant enzyme levels in the plasma of control and STZ-diabetic rats.

	Group I	Group II	Group III	Group IV	Group V
TBARS	2.7 ± 0.01	5.21 ± 0.40	3.42 ± 0.23	3.03 ± 0.63*	2.89 ± 0.04
HP	8.42 ±0.30	18.32 ±0.04	13.03 ±3.08	11.26±1.49*	9.32 ±0.13
SOD	23.58±0.57	10.87 ±0.88	16.99 ± 1.79	18.76±2.27*	20.92 ±1.37
CAT	2.32 ±0.33	1.58 ±0.13	1.97 ±0.36	2.12 ±0.08*	2.23±0.52
GPx	2.62 ±0.26	4.84 ±0.62	3.05 ±0.53	2.72 ±0.41*	2.89 ±0.32

Values are expressed as mean ± S.D (n=6) statistically significant alterations are expressed as $p<0.05$.* Group III and IV and V are compared with group II.
values of HP Values X 10^{-5} Mm/dl), TBARS(n moles / ml), SOD, CAT and GPx (U/ml)

HISTOPATHOLOGICAL STUDIES

Pancreas: The section of control rat showed normal architecture with islet of Langerhans which formed of numerous compactly arranged cells and diabetic rat showed pyknotic nuclei and dark nuclei, and few cells at the periphery had round or ovoid nuclei (Figure 6, 7), where as section of rat treated with *COLF* 100 mg/kg b.w showed shrunken nuclei and mild inflammatory cells (Figure 8). Rats treated with *COLF*200 mg/kg bw showed normal architecture with shrunken nuclei (Figure 9). Section of rat treated with Glibenclamide showed islet cells with vascular nuclei and pyknotic nuclei (Figure 10).

DISCUSSION

DM is currently one of the most costly and burdensome chronic diseases and is a condition that is increasing in epidemic proportions throughout the world[27]. According to WHO, around 171 million people worldwide were suffering from diabetes in 2000 and this figure is predicted to double by 2030 [28, 29].

Oral hypoglycemic agents have been used to control type 2 diabetes, and many of them act by stimulating insulin secretion even in the presence of low glucose concentrations, with the consequent risk of producing hypoglycemia, one of the most undesirable side effects of treating diabetes with oral agents such as sulphonylureas, which cause disruption in insulin action.[30] It is also possible that treatment with *COLMF* could be facilitating utilization of glucose by peripheral tissues. This mechanism has been observed in the Nymphaea stellata[31] *Cassia auriculata* Linn, [32] *Cocculus hirsutus* [33] in the treatment of DM.

Hyperglycemia has an important role in the pathogenesis of long-term complications, besides, during diabetes, the excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in diabetic rats[34]. In uncontrolled diabetes, there is an increased glycosylation of hemoglobin, which has been found to be increased over a long period of time in diabetes.[35] Therefore, measurement of HbAlc is supposed to be very sensitive index for glycemic control. Glycosylated hemoglobin was significantly increased in diabetic animals, and this increase was found directly proportional to the fasting blood glucose level.[36] Oral administration of *COLMF* significantly and dose dependently restored the Hb, HbAlc levels near to normal.

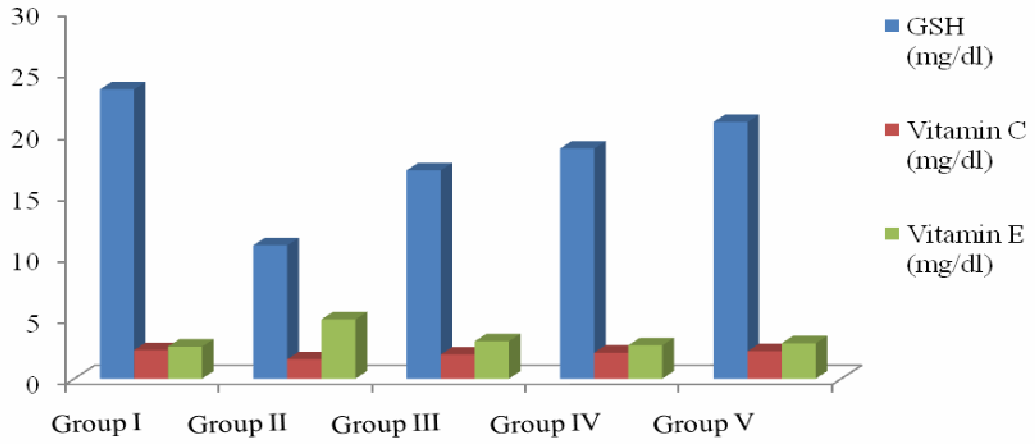
Diabetes mellitus is also associated with a marked decrease in the level of liver glycogen[37]. In this context, glycogen content of liver markedly decreases in diabetes and this alteration is normalized by insulin treatment[38]. Additionally, Witters and Auruch[39] have reported that administration of insulin increased the activity of hepatic glycogen. Oral administration of *COLMF* significantly and dose dependently increases hepatic glycogen levels dose dependently in STZ-diabetic rats.

A significant antihyperglycemia action on the fasting blood glucose and plasma insulin was observed after 45 days treatment of diabetic rats with different doses of *COLMF*. The hypoglycemic effect of *COLMF* in diabetic rats was more powerful, suggesting that it could be caused by an increase in peripheral glucose consumption. It appears that still insulin producing cells are functioning and the stimulation of insulin release could be responsible for most of the metabolic effects. In this context, leaves of *Gymnema sylvestre* and *Andrographis paniculata Nees* exhibited potent hypoglycemic activity in experimental animal models of diabetes.[40, 41]

It is well known that diabetes mellitus is associated with an increased production of reactive oxygen species and a reduction in anti oxidative defenses. This defense includes the enzymes SOD, CAT and GPx[42]. The diabetogenic action of STZ can be prevented by the superoxide dismutase and catalase, hence there is evidence to suggest that the

incidence of diabetes involves superoxide anion and hydroxyl radicals[43]. The enzyme SOD scavenges superoxide radicals (O_2^-) by catalysing the conversion of two of these radicals into hydrogen peroxide and molecular oxygen[44]. Earlier workers had reported a decrease in the activities of these antioxidant enzymes (SOD, CAT and GPx) in the plasma and liver of diabetic rats[45]. We also found that the activities of SOD and CAT in untreated diabetic control animals were significantly ($p < 0.01$) lower than the normal control animals. The result of increased activities of SOD and CAT suggests that *COLMF* contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of O_2^{--} and OH^{--}. In the present investigation *COLMF* dose dependently decreased the levels of antioxidant enzymes in plasma and liver tissues of diabetic rats by reducing the oxidative stress due to its potential antioxidant activity. The promising antioxidant and antihyperglycemia efficacies of *COLMF* demonstrated in this study may open new avenues in the treatment of diabetes and its complications.

Figure 4: Effect of *COLMF* on Non-enzymatic Antioxidant levels in STZ-diabetic rats



Values are expressed as mean ± S.D (n=6). Statistically significant alterations are expressed as *p<0.05. Group III and IV and V are compared with group II.

Histopathological observation of normal and experimental rat pancreas (H&E, 400).

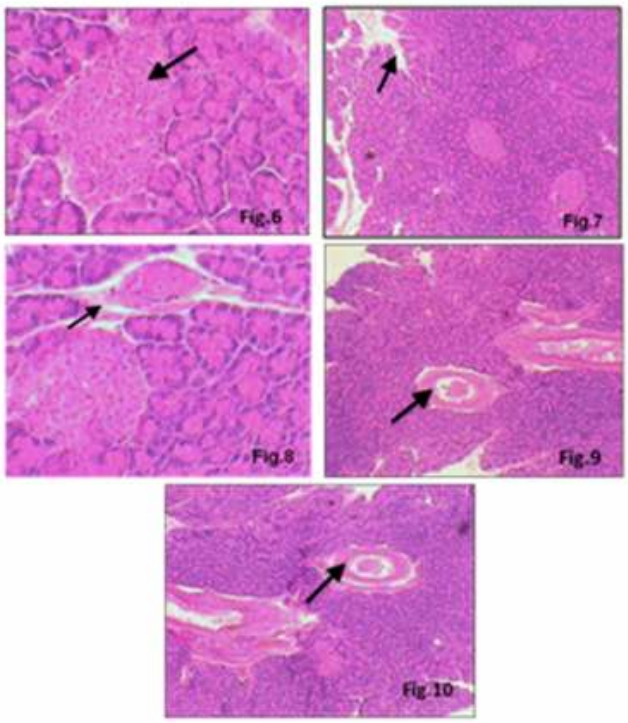


Fig. 6. Control group showing normal architecture of pancreatic islets

Fig. 7. diabetic control group showing pancreatic acini, small atrophic islet cells

Fig. 8. *COLMF* 100 mg/kg bw. treated group showing mild expansion pancreatic islets

Fig. 9. *COLMF* 200 mg/kg bw. treated group showing moderate expansion pancreatic islets and prominent hyperplastic islet

Fig. 10. Glibenclamide treated group showing normal hyperplastic of islets

ACKNOWLEDGEMENTS

I gratefully acknowledge Dr. V Kalyan Chakravarthy, M.D Pathology, Siddhardha Medial College, Vijayawada, for his involvement in histopathological examination and interpretation

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